



**U.S. Army  
Environmental  
Center**

**DEMONSTRATION PLAN  
FOR  
PHYTOREMEDIATION OF EXPLOSIVES  
CONTAMINATED GROUNDWATER IN  
CONSTRUCTED WETLANDS**

**at  
MILAN ARMY AMMUNITION PLANT  
Milan, Tennessee**

**Volume II of II**

*Prepared for*  
**U.S. ARMY ENVIRONMENTAL CENTER  
Aberdeen Proving Ground, Maryland 21010-5401**

*Funded Through*



**U.S. Department of Defense  
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*Prepared by*  
**Tennessee Valley Authority  
Environmental Research Center  
Muscle Shoals, Alabama 35660-1010**

**January 1996**

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Report No. SFIM-AEC-ET-CR-95090**

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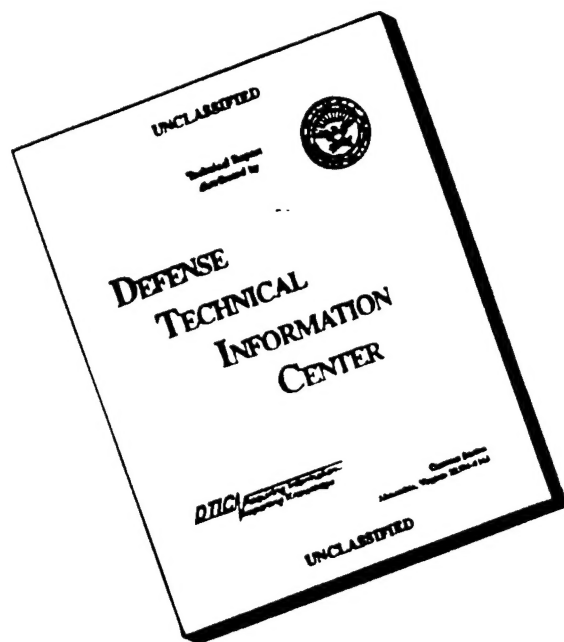
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Phytoremediation Demo. Plan

Milan AAP



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***Demonstration Plan***  
**For Phytoremediation of Explosives-Contaminated Groundwater  
In Constructed Wetlands  
at Milan Army Ammunition Plant**

**Volume II of II**

***Prepared for***  
**U.S. Army Environmental Center  
Environmental Technology Division  
Aberdeen Proving Ground, MD 21010-5401  
POC: Ms. Darlene Bader**

***Funded Through***  
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***Prepared by***  
**Tennessee Valley Authority  
Environmental Research Center**

**January 1996**



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## ABBREVIATIONS

AAP	-Army Ammunition Plant
AFUDC	-Allowance for Funds Used During Construction
ALEA	-Analytical Laboratory of Environmental Applications
BOD	-Biochemical Oxygen Demand
CE	-Capillary Electrophoresis
COD	-Chemical Oxygen Demand
DANT	-Diaminonitrotoluene
DNT	-Dinitrotoluene
DO	-Dissolved Oxygen
DoD	-Department of Defense
EC	-Electrical Conductivity
ECWTP	-Explosive-Contaminated Wastewater Treatment Plants
EPA	-Environmental Protection Agency
ESTCP	-Environmental Security Technology Certification Program
FIA	-Flow Injection Analyzer
GAC	-Granular Activated Carbon
GOCO	-Government Owned Contractor Operated
gpm	-Gallons per Minute
HPLC	-High Performance Liquid Chromatography
IC	-Ion Chromatography
ICP	-Inductively Coupled Plasma
HMX	-Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
LAP	-Load, Assemble, Pack
LMOS	-Lockheed Martin Ordnance Systems
M	-Million
MAAP	-Milan Army Ammunition Plant
MDL	-Method Detection Limit
MEV	-Million Electron Volt
MMOS	-Martin Marietta Ordnance Systems, Inc.
MOD	-Milan Ordnance Depot
NH <sub>4</sub> -N	-Ammonia Nitrogen
NO <sub>3</sub>	-Nitrate
(NO <sub>3</sub> +NO <sub>2</sub> )-N	-Nitrate + Nitrite Nitrogen
NPDES	-National Pollutant Discharge Elimination System
PO <sub>4</sub>	-Orthophosphate
PO <sub>4</sub> -P	-Orthophosphate - Phosphorus
PVC	-Poly Vinyl Chloride
QA	-Quality Assurance
QC	-Quality Control
RDX	-Hexahydro-1,3,5-trinitro-1,3,5-triazine



R&D	-Research and Development
STP	-Sewage Treatment Plant
TAT	-Triaminotoluene
TKN	-Total Kjeldahl Nitrogen
TNB	-Trinitrobenzene
TNT	-2,4,6 Trinitrotoluene
TOC	-Total Organic Carbon
TVA	-Tennessee Valley Authority
USAEC	-U.S. Army Environmental Center
USEPA	-United States Environmental Protection Agency
UT	-University of Tennessee
UV	-Ultraviolet
WCOP	-Wolf Creek Ordnance Plant
WES	-Waterways Experiment Station



**APPENDIX B**

**METHODS AND PROCEDURES**



**Appendix B-1 – METHOD 8330: Nitroaromatics and Nitramines by High  
Performance Liquid Chromatograph (HPLC)**



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<b>Documents</b>
<b>METHOD 8330: Nitoaromatics and Nitramines by High Performance Liquid Chromatograph (HPLC)</b>



## METHOD 8330

### NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

#### 1.0 SCOPE AND APPLICATION

1.1 Method 8330 is intended for the trace analysis of explosives residues by high performance liquid chromatography using a UV detector. This method is used to determine the concentration of the following compounds in a water, soil, or sediment matrix:

Compound	Abbreviation	CAS No <sup>a</sup>
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
2-Amino-4, 6-dinitrotoluene	2-Am-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

a Chemical Abstracts Service Registry number

1.2 Method 8330 provides a salting-out extraction procedure for low concentration (parts per trillion, or nanograms per liter) of explosives residues in surface or ground water. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration (See Table 1).

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See NOTE in Sec. 5.3.1 and Sec. 11 on Safety.

1.4 The estimated quantitation limits (EQLs) of target analytes determined by Method 8330 in water and soil are presented in Table 1.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials. (See Sec. 11.0



on SAFETY.) Each analyst must demonstrate the ability to generate acceptable results with this method.

## 2.0 SUMMARY OF METHOD

2.1 Method 8330 provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosives residues in water, soil and sediment matrix. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-Level Salting-out Method With No Evaporation: Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent grade water. An aliquot is separated on a C-18 reverse phase column, determined at 254 nm, and confirmed on a CN reverse phase column.

2.3 High-level Direct Injection Method: Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reverse phase column, determine at 254 nm, and confirmed on a CN reverse phase column. If HMX is an important target analyte, methanol is preferred.

2.4 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed as in Sec. 2.3.

## 3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

3.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

3.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.



## 4.0 APPARATUS AND MATERIALS

### 4.1 HPLC system

4.1.1 HPLC - equipped with a pump capable of achieving 4000 psi, a 100  $\mu$ l loop injector and a 254 nm UV detector (Perkin Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of a stable baseline at 0.001 absorbance units full scale.

#### 4.1.2 Recommended Columns:

4.1.2.1 Primary column: C-18 Reverse phase HPLC column, 25 cm x 4.6 mm (5  $\mu$ m), (Supelco LC-18, or equivalent).

4.1.2.2 Secondary column: CN Reverse phase HPLC column, 25 cm x 4.6 mm (5  $\mu$ m), (Supelco LC-CN, or equivalent).

#### 4.1.3 Strip chart recorder.

#### 4.1.4 Digital integrator (optional).

#### 4.1.5 Autosampler (optional).

### 4.2 Other Equipment

#### 4.2.1 Temperature controlled ultrasonic bath.

#### 4.2.2 Vortex mixer.

#### 4.2.3 Balance, $\pm$ 0.0001 g.

#### 4.2.4 Magnetic stirrer with stirring pellets.

4.2.5 Water bath - Heated, with concentric ring cover, capable of temperature control ( $\pm$  5°C). The bath should be used in a hood.

#### 4.2.6 Oven - Forced air, without heating.

### 4.3 Materials

4.3.1 High pressure injection syringe - 500  $\mu$ L, (Hamilton liquid syringe or equivalent).

#### 4.3.2 Disposable cartridge filters - 0.45 $\mu$ m Teflon filter.

#### 4.3.3 Pipets - Class A, glass, Appropriate sizes.

#### 4.3.4 Pasteur pipets.

#### 4.3.5 Scintillation Vials - 20 mL, glass.

#### 4.3.6 Vials - 15 mL, glass, Teflon-lined cap.



4.3.7 Vials- 40 mL, glass, Teflon-lined cap.

4.3.8 Disposable syringes - Plastipak, 3 mL and 10 mL or equivalent.

4.3.9 Volumetric flasks - Appropriate sizes with ground glass stoppers, Class A.

NOTE: The 100 mL and 1 L volumetric flasks used for magnetic stirrer extraction must be round.

4.3.10 Vacuum desiccator - Glass.

4.3.11 Mortar and pestle - Steel.

4.3.12 Sieve - 30 mesh.

4.3.13 Graduated cylinders - Appropriate sizes.

#### 4.4 Preparation of Materials

4.4.1 Prepare all materials to be used as described in Chapter 4 for semivolatile organics.

### 5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.1.1 Acetonitrile,  $\text{CH}_3\text{CN}$  - HPLC grade.

5.1.2 Methanol,  $\text{CH}_3\text{OH}$  - HPLC grade.

5.1.3 Calcium chloride,  $\text{CaCl}_2$  - Reagent grade. Prepare an aqueous solution of 5 g/L.

5.1.4 Sodium chloride,  $\text{NaCl}$ , shipped in glass bottles - reagent grade.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

#### 5.3 Stock Standard Solutions

5.3.1 Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100 mL volumetric flask and dilute to volume with acetonitrile. Invert flask several times until dissolved. Store in



refrigerator at 4°C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

NOTE: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See SAFETY in Sec. 11 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature requires several days. DO NOT DRY AT HEATED TEMPERATURES!

#### 5.4 Intermediate Standards Solutions

5.4.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

5.4.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

5.4.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

#### 5.5 Working standards

5.5.1 Calibration standards at a minimum of five concentration levels should be prepared through dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L calcium chloride solution (Sec. 5.1.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

#### 5.6 Surrogate Spiking Solution

5.6.1 The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

#### 5.7 Matrix Spiking Solutions

5.7.1 Prepare matrix spiking solutions in methanol such that the concentration in the sample is five times the Estimated Quantitation Limit (Table 1). All target analytes should be included.



## 5.8 HPLC Mobile Phase

5.8.1 To prepare 1 liter of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

## 6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Follow conventional sampling and sample handling procedures as specified for semivolatile organics in Chapter Four.

6.2 Samples and sample extracts must be stored in the dark at 4°C. Holding times are the same as for semivolatile organics.

## 7.0 PROCEDURE

### 7.1 Sample Preparation

7.1.1 Aqueous Samples: It is highly recommended that process waste samples be screened with the high-level method to determine if the low level method (1-50 µg/L) is required. Most groundwater samples will fall into the low level method.

#### 7.1.1.1 Low-Level Method (salting-out extraction)

7.1.1.1.1 Add 251.3 g of sodium chloride to a 1 L volumetric flask (round). Measure out 770 mL of a water sample (using a 1 L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

7.1.1.1.2 Add 164 mL of acetonitrile (measured with a 250 mL graduated cylinder) while the solution is being stirred and stir for an additional 15 minutes. Turn off the stirrer and allow the phases to separate for 10 minutes.

7.1.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100 mL volumetric flask (round). Add 10 mL of fresh acetonitrile to the water sample in the 1 L flask. Again stir the contents of the flask for 15 minutes followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

7.1.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100 mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 minutes, followed by 10 minutes for phase separation. Carefully transfer the acetonitrile phase



to a 10 mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

7.1.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100 mL volumetric flask. Again stir the contents of the flask for 15 minutes, followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 10 mL graduated cylinder (transfer to a 25 mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [ $V_t$ ] in the calculation of concentration after converting to  $\mu\text{L}$ ). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

7.1.1.1.6 If the diluted extract is turbid, filter it through a 0.45 -  $\mu\text{m}$  Teflon filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

#### 7.1.1.2 High-Level Method

7.1.1.2.1 Sample filtration: Place a 5 mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45- $\mu\text{m}$  Teflon filter using a disposable syringe. Discard the first 3 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

#### 7.1.2 Soil and Sediment Samples

7.1.2.1 Sample homogenization: Dry soil samples in air at room temperature or colder to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30 mesh sieve.

NOTE: Soil samples should be screened by Method 8515 prior to grinding in a mortar and pestle (See Safety Sec. 11.2).

#### 7.1.2.2 Sample extraction

7.1.2.2.1 Place a 2.0 g subsample of each soil sample in a 15 mL glass vial. Add 10.0 mL of acetonitrile, cap with



Teflon-lined cap, vortex swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

7.1.2.2.2 After sonication, allow sample to settle for 30 minutes. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 5.1.3) in a 20 mL vial. Shake, and let stand for 15 minutes.

7.1.2.2.3 Place supernatant in a disposable syringe and filter through a 0.45- $\mu$ m Teflon filter. Discard first 3 mL and retain remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

## 7.2 Chromatographic Conditions (Recommended)

Primary Column: C-18 reverse phase HPLC column, 25-cm x 4.6-mm, 5  $\mu$ m, (Supelco LC-18 or equivalent).

Secondary Column: CN reverse phase HPLC column, 25-cm x 4.6-mm, 5  $\mu$ m, (Supelco LC-CN or equivalent).

Mobile Phase: 50/50 (v/v) methanol/organic-free reagent water.

Flow Rate: 1.5 mL/min

Injection volume: 100- $\mu$ L

UV Detector: 254 nm

## 7.3 Calibration of HPLC

7.3.1 All electronic equipment is allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 20 min at 1.5 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

7.3.2 Initial Calibration. Injections of each calibration standard over the concentration range of interest are made sequentially into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Experience indicates that a linear calibration curve with zero intercept is appropriate for each analyte. Therefore, a response factor for each analyte can be taken as the slope of the best-fit regression line.

7.3.3 Daily Calibration. Analyze midpoint calibration standards, at a minimum, at the beginning of the day, singly at the midpoint of the run, and singly after the last sample of the day (assuming a sample group of 10 samples or less). Obtain the response factor for each analyte from the mean peak heights or peak areas and compare it with the response factor obtained for the initial calibration. The mean response factor for the



daily calibration must agree within  $\pm 15\%$  of the response factor of the initial calibration. The same criteria is required for subsequent standard responses compared to the mean response of the triplicate standards beginning the day. If this criterion is not met, a new initial calibration must be obtained.

#### 7.4 HPLC Analysis

7.4.1 Analyze the samples using the chromatographic conditions given in Sec. 7.2. All positive measurements observed on the C-18 column must be confirmed by injection onto the CN column.

7.4.2 Follow Sec. 7.0 in Method 8000 for instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 10 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.4.3 Table 2 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1.

7.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

7.4.5 Calculation of concentration is covered in Sec. 7.0 of Method 8000.

#### 8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. Quality control to validate sample extraction is covered in Method 3500.

8.2 Quality control required to validate the HPLC system operation is found in Method 8000, Sec. 8.0.

8.3 Prior to preparation of stock solutions, acetonitrile, methanol, and water blanks should be run to determine possible interferences with analyte peaks. If the acetonitrile, methanol, or water blanks show contamination, a different batch should be used.

#### 9.0 METHOD PERFORMANCE

9.1 Table 3 presents the single laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.



9.2 Table 4 presents the multilaboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.

9.3 Table 5 presents the multilaboratory variance of the high concentration method for water based on data from nine laboratories.

9.4 Table 6 presents multilaboratory recovery data from the analysis of spiked soil samples by seven laboratories.

9.5 Table 7 presents a comparison of method accuracy for soil and aqueous samples (high concentration method).

9.6 Table 8 contains precision and accuracy data for the salting-out extraction method.

## 10.0 REFERENCES

1. Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USA Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.
2. Grant, C.L., A.D. Hewitt and T.F. Jenkins (1989) Comparison of low concentration measurement capability estimates in trace analysis: Method Detection Limits and Certified Reporting Limits. USA Cold Regions Research and Engineering Laboratory, Special Report 89-20.
3. Jenkins, T.F., C.F. Bauer, D.C. Leggett and C.L. Grant (1984) Reversed-phased HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater. USA Cold Regions Research and Engineering Laboratory, CRREL Report 84-29.
4. Jenkins, T.F. and M.E. Walsh (1987) Development of an analytical method for explosive residues in soil. USA Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.
5. Jenkins, T.F., P.H. Miyares and M.E. Walsh (1988a) An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 88-23.
6. Jenkins, T.F. and P.H. Miyares (1992) Comparison of Cartridge and Membrane Solid-Phase Extraction with Salting-out Solvent Extraction for Preconcentration of Nitroaromatic and Nitramine Explosives from Water. USA Cold Regions Research and Engineering Laboratory, Draft CRREL Special Report.
7. Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1988b) Development of an analytical method for the determination of explosive residues in soil. Part II: Further development and ruggedness testing. USA Cold Regions Research and Engineering Laboratory, CRREL Report 88-8.



8. Leggett, D.C., T.F. Jenkins and P.H. Miyares (1990) Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water. Analytical Chemistry, 62: 1355-1356.
9. Miyares, P.H. and T.F. Jenkins (1990) Salting-out solvent extraction for determining low levels of nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 90-30.

## 11.0 SAFETY

11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by Method 8330. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Follow the note for drying the neat materials at ambient temperatures.

11.2 It is advisable to screen soil or waste samples using Method 8515 to determine whether high concentrations of explosives are present. Soil samples as high as 2% 2,4,6-TNT have been safely ground. Samples containing higher concentrations should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, however, the other nitroaromatics will also cause a color to be developed and provide a rough estimation of their concentrations. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.



TABLE 1  
ESTIMATED QUANTITATION LIMITS

Compounds	Water ( $\mu\text{g/L}$ )		Soil (mg/kg)
	Low-Level	High-Level	
HMX	-	13.0	2.2
RDX	0.84	14.0	1.0
1,3,5-TNB	0.26	7.3	0.25
1,3-DNB	0.11	4.0	0.25
Tetryl	-	4.0	0.65
NB	-	6.4	0.26
2,4,6-TNT	0.11	6.9	0.25
4-Am-DNT	0.060	-	-
2-Am-DNT	0.035	-	-
2,6-DNT	0.31	9.4	0.26
2,4-DNT	0.020	5.7	0.25
2-NT	-	12.0	0.25
4-NT	-	8.5	0.25
3-NT	-	7.9	0.25



TABLE 2  
RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

Compound	Retention time (min)		Capacity factor (k)*	
	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

\* Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.



TABLE 3  
SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
Tetryl	17	3.1	17.9	2.3	0.41	18.0
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3



TABLE 4  
MULTILABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14	3.7	26.0
				153	37.3	24.0
RDX	60	2.6	4.4	104	17.4	17.0
				877	67.3	7.7
1,3,5-TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
Tetryl	17	5.22	30.7	2.3	0.49	21.3
2,4,6-TNT	40	1.88	4.7	7.0	1.27	18.0
				669	63.4	9.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0

TABLE 5  
MULTILABORATORY VARIANCE OF METHOD FOR WATER SAMPLES<sup>a</sup>

Compounds	Mean Conc. ( $\mu$ g/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

<sup>a</sup> Nine Laboratories



TABLE 6  
MULTILABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

Laboratory	Concentration ( $\mu\text{g/g}$ )						
	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std Dev	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

\* Between true value and mean determined value.



TABLE 7  
COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES  
(HIGH CONCENTRATION METHOD)

Analyte	Recovery (%)	
	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

\* Taken from Bauer et al. (1989), Reference 1.

\*\* Taken from Jenkins et al. (1984), Reference 3.



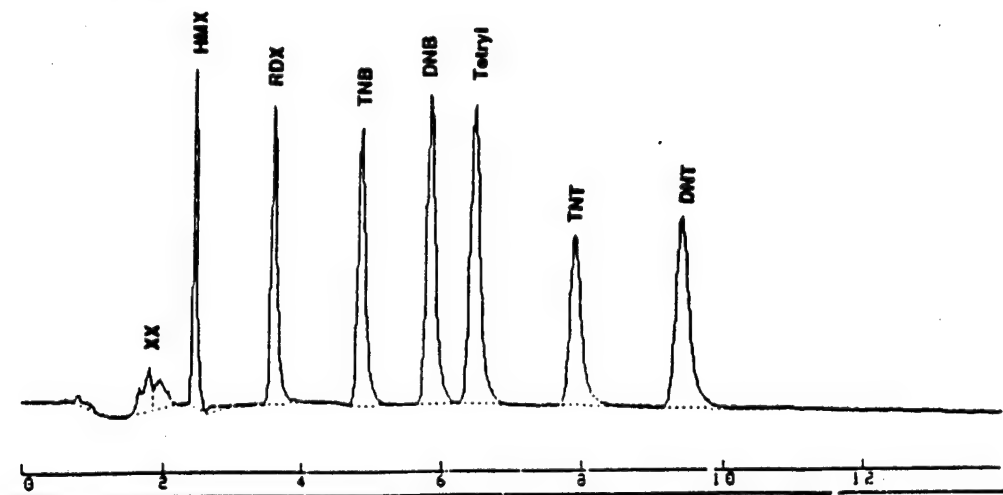
TABLE 8  
PRECISION AND ACCURACY DATA FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	No. of Samples <sup>1</sup>	Precision (% RSD)	Ave. Recovery (%)	Conc. Range ( $\mu\text{g/L}$ )
HMX	20	10.5	106	0-1.14
RDX	20	8.7	106	0-1.04
1,3,5-TNB	20	7.6	119	0-0.82
1,3-DNB	20	6.6	102	0-1.04
Tetryl	20	16.4	93	0-0.93
2,4,6-TNT	20	7.6	105	0-0.98
2-Am-DNT	20	9.1	102	0-1.04
2,4-DNT	20	5.8	101	0-1.01
1,2-NT	20	9.1	102	0-1.07
1,4-NT	20	18.1	96	0-1.06
1,3-NT	20	12.4	97	0-1.23

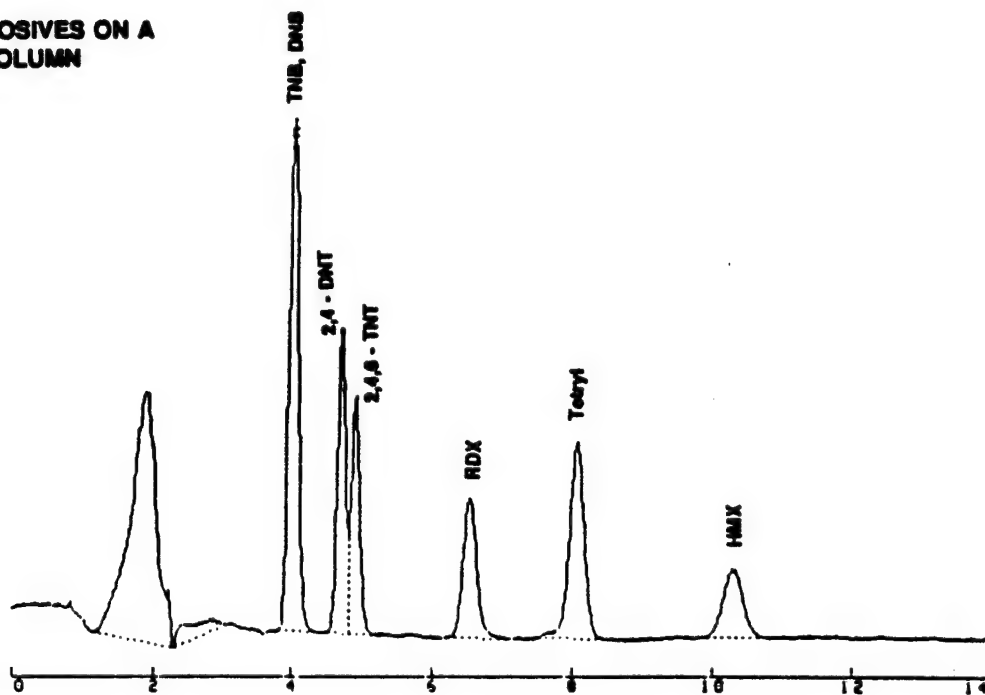
<sup>1</sup>Reagent water



**EXPLOSIVES ON A  
C18 COLUMN**



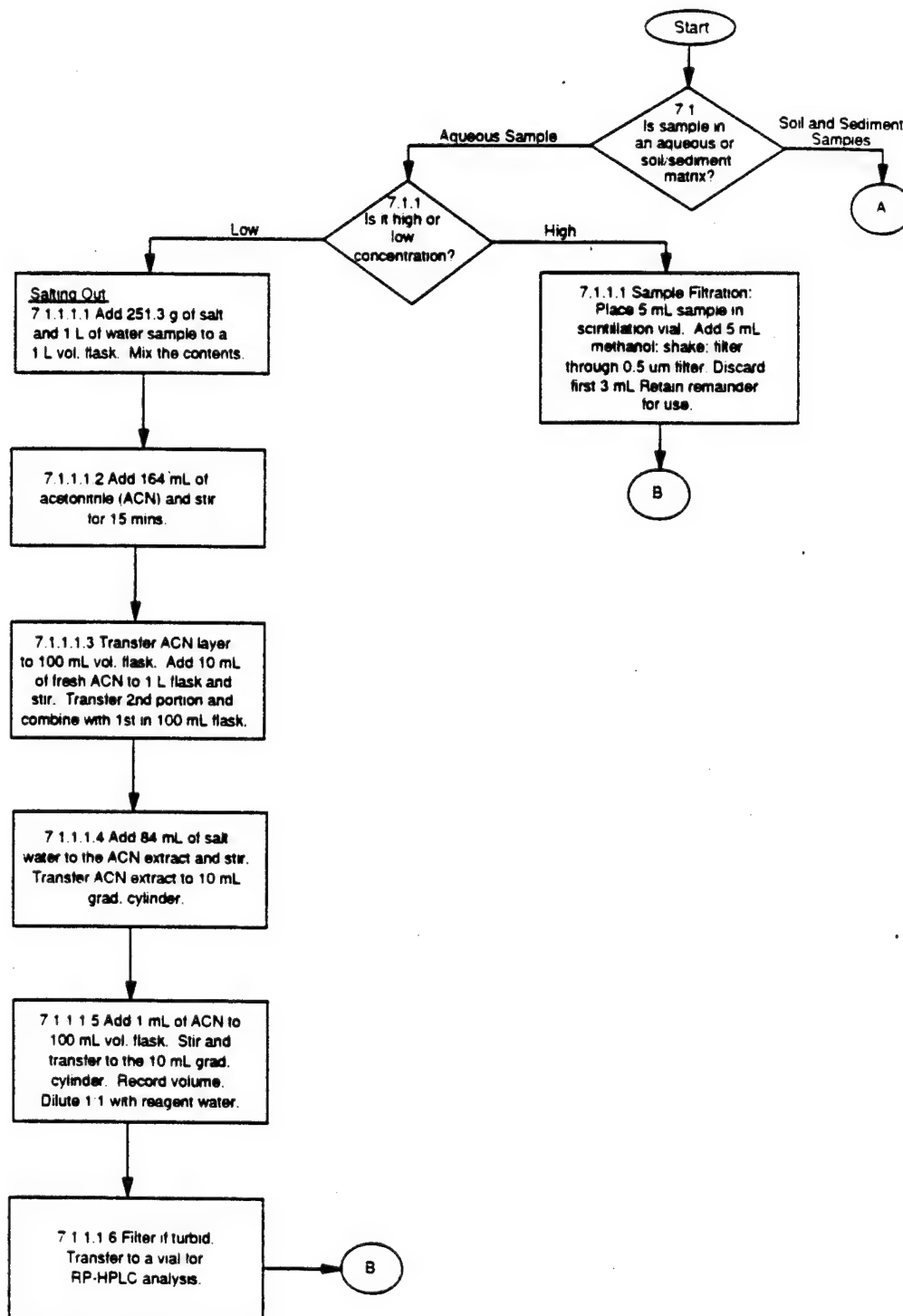
**EXPLOSIVES ON A  
CN COLUMN**



**FIGURE 1**  
CHROMATOGRAMS FOR COLUMNS DESCRIBED IN Sec. 4.1.2.  
COURTESY OF U.S. ARMY CORPS OF ENGINEERS, OMAHA, NE.

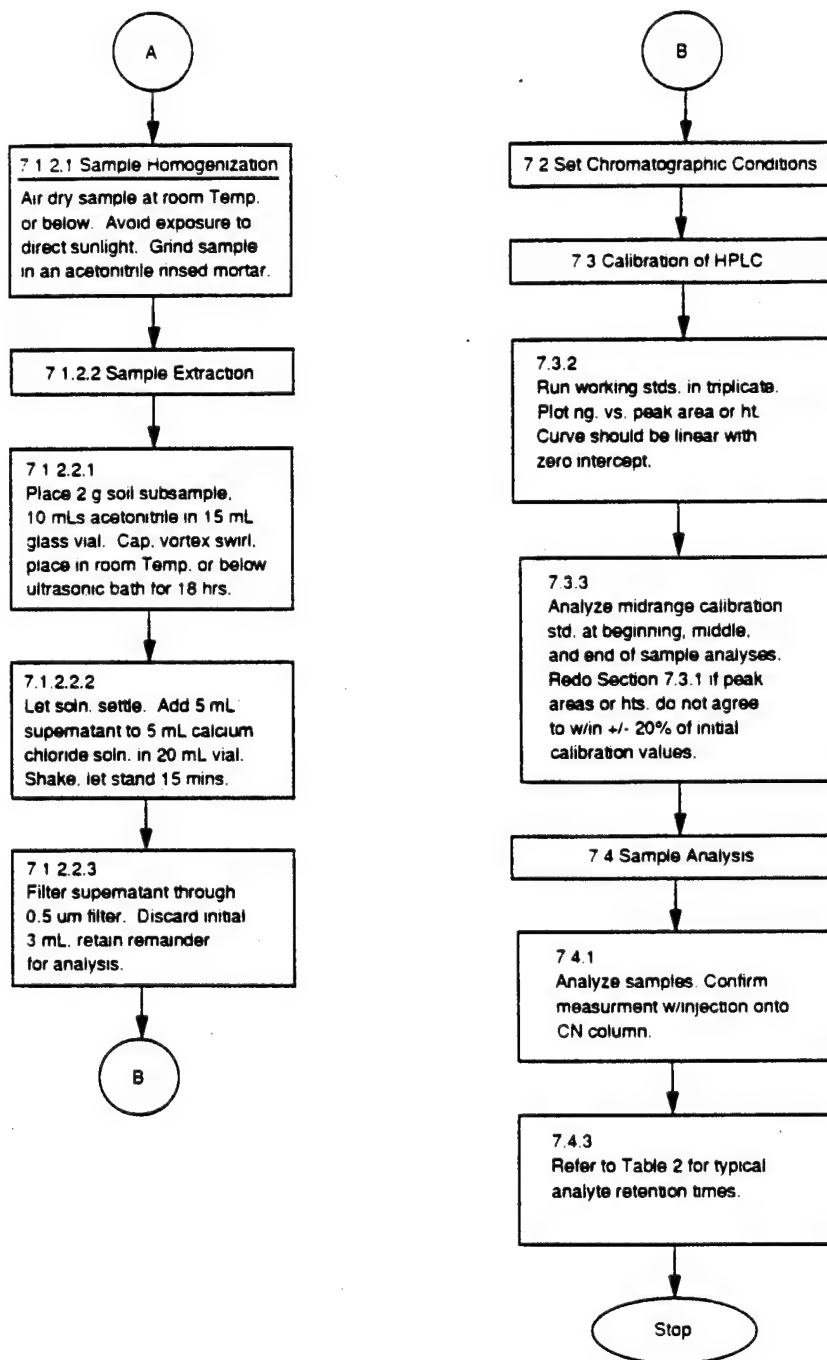


METHOD 8330  
NITROAROMATICS AND NITRAMINES BY HIGH  
PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)





METHOD 8330  
(continued)





## Method 8330 - Addendum A

This Addendum serves to describe our interpretation of certain portions of Method 8330 and to describe curve fitting as actually performed.

1. Interpretation: Midpoint calibration standards are run in triplicate at the start of the run and every ten samples thereafter (and as the last sample of the run). These are counted as they are loaded on the machine. A pair of reagent blanks are usually run before the three initial midpoint calibration standards. For every twenty customer samples, one is selected to have an aliquot run with a matrix spike added and another aliquot run with a matrix spike duplicate. In addition, for every twenty customer samples, a method blank is run. Thus, an analytical run can be summarized as on the following page.

Here, "Reagent Blank" refers to only the solvents such as are used to make calibration solutions. "Method Blank" would include solvent carried through any preparation or extraction steps associated with the batch. The "Laboratory Control Sample" may be called by other names in various documents. It is made using standard material other than that used in calibrating the machine. Note also that the customer sample to be spiked as the matrix spike and matrix spike duplicate may be chosen at random through any group of 20.

The "Midpoint Calibration Standard" may be present as several individual samples rather than as a single sample containing all analytes.



i	Reagent Blank
ii	Reagent Blank
iii	Midpoint Cal. Std.
iv	Midpoint Cal. Std.
v	Midpoint Cal. Std.
1	Method Blank
2	Laboratory Control Sample
3	Customer Sample 1
4	Customer Sample 2
5	Customer Sample 3
6	Customer Sample 4
7	Customer Sample 5
8	Matrix Spike of Sample 5
9	Matrix Spike Duplicate of 5
10	Customer Sample 6
	Midpoint Cal. Std.
11	Customer Sample 7
12	Customer Sample 8
13	Customer Sample 9
14	Customer Sample 10
15	Customer Sample 11
16	Customer Sample 12
17	Customer Sample 13
18	Customer Sample 14
19	Customer Sample 15
20	Customer Sample 16
	Midpoint Cal. Std.
21	Customer Sample 17
22	Customer Sample 18
23	Customer Sample 19
24	Customer Sample 20
25	Method Blank
26	Laboratory Control Sample
27	Customer Sample 21
28	Matrix Spike of 21
29	Matrix Spike Duplicate of 21
30	Customer Sample 22
	Midpoint Cal. Std.
31	Customer Sample 23
32	Customer Sample 24
etc.	

2. Modification: If any midpoint calibration standard does not fall within 15% of the daily average of the first three midpoint calibration standards, then the previous and following ten samples will be reanalyzed.

3. Interpretation: Calibration curves will be run and utilized as described in Method 8330. Curves will be run based on peak height rather than peak area. Curve fits will be forced through the origin utilizing standard peak fitting software provided by the manufacturer of the chromatography system or a commercially available spreadsheet.



4. Interpretation: Calibration curves will be run initially. These curves will be placed into use and will be used until there is indication of a change in system response. They will not be re-run unless the midpoint calibration checks indicate that the response slope of the system has changed significantly. There is no need to re-run the curves monthly or on any other frequency unless there is indication of a change. Furthermore, the solutions may be used longer than 30 days as quality control check samples since they can be compared to their initial concentration. We intend to keep them in use until a change is indicated.

5. Modification: The calcium chloride in Step 5.5 "Working Standards" will be replaced with HPLC grade water when analyzing acetonitrile extracts or HPLC grade acetonitrile when analyzing water samples. (The calcium chloride solution is present to coagulate clay particles in soil analysis.)

6. Modification: Since the site has been characterized well, we will not be utilizing the dual-column confirmation analysis mentioned in steps 2.3, 4.1.2.2, 7.2, and 7.4.1.

7. Modification: We are not using surrogates as mentioned in step 5.6.

8. Modification: Our spiking solutions are in acetonitrile or water, not methanol as mentioned in 5.7.

9. Modification: We are not premixing the mobile phase as specified in step 5.8.1 but are mixing the reagents in the pump.



10. Modification: Steps in Section 7.1 "Sample Preparation" will be replaced with the following

7.1.1.1 - Not used at present.

7.1.1.2 Treat samples as in Addendum B

7.1.2 Soil and Sediment Samples - This section is used for preparation of plant and gravel samples.

7.1.2.1 - Follow this step for samples which are not already dry when received. Gravel samples are not ground.

7.1.2.2 Sample Extraction

7.1.2.2.1 We weigh out the sample, add 100 ml of acetonitrile, add a stir bar and stir on medium speed for at least one hour.

7.1.2.2.2 Replace the calcium chloride solution with water.



Method 8330 - Addendum B  
Sample Preparation

1.0 Sample Types

1.1 Samples are one of two types:

1.1.1 Water samples

1.1.2 Acetonitrile from the extraction of solids (gravel or plant matter).

2.0 Before sample preparation starts, place all samples in a dark area and allow them to reach room temperature.

3.0 Sample Preparation

3.1 Combine acetonitrile matrices 1:1 with water (usually 2 mL of sample plus 2 mL of water) in an 8 mL vial. Cap the vial and cover it with an aluminum foil sleeve. Shake it briefly, and then place it in a dark area for a minimum of 20 minutes.

3.2 Combine water matrices 1:1 with acetonitrile (usually 2 mL of sample plus 2 mL of acetonitrile) in an 8 mL vial. Cap the vial and cover it with an aluminum foil sleeve. Shake it briefly, and then place it in a dark area for a minimum of 20 minutes.

6.0 Filtration

6.1 Pour the contents of each 8 mL vial into a 10 mL syringe whose plunger has been removed and to which is attached a 25 mm syringe filter.

6.2 Remount the syringe plunger and filter approximately 2 mL of solution into a waste breaker.

6.3 Filter a portion of the remaining solution into an amber autosampler vial.

6.4 Keep the autosampler vials in a dark area until they are loaded on the Varian model 9100 autosampler.



Method 8330 - Addendum C  
Quality Control

1.0 Field QC

Sample sets received from the Milan test site may contain a set of field quality control samples for every 20 samples consisting of a blank solution and a field QC solution. Count these the same as other field samples in determining batch size.

2.0 Laboratory QC

Once in the laboratory, add the following list of quality control sample types for every 20 analytical samples: method blank, laboratory control sample, matrix spike, and matrix spike duplicate.

3.0 Method Blank

3.1 For liquid samples, use a 10 mL portion of acetonitrile treated as a normal analytical liquid sample.

3.2 For solid samples, place a clean sample of substrate in a precleaned 250 mL jar along with a stir bar and 100 mL of acetonitrile.

3.3 Treat the sample as a normal analytical sample.

4.0 Laboratory Control Sample

4.1 The spike solution must be prepared from a source separate from that used for calibration standards. Match the spike solution to the analytes being studied (TNT, RDX, or both TNT and RDX.). The spiking solution concentration should be made up to approximately 10 ug/mL for each analyte.

4.2 For liquid samples, add 0.5 mL of spike solution to a 10 mL volumetric flask. Bring it to volume with acetonitrile.

4.3 For solid samples, place a clean sample of substrate in a precleaned 250 mL jar along with a stir bar. Pipette 5 mL of spike solution onto the substrate. Add 95 mL of acetonitrile measured in a graduated cylinder.

4.4 Treat the sample as a normal analytical sample.

5.0 Matrix Spike and Matrix Spike Duplicate

5.1 Select a routine field sample (not a field QC sample, sacrifice or stock solution) at random to be spiked.

5.2 Match the spiking solution to the analytes being studied. The spiking solution should be made up to approximately 10 ug/mL for each analyte.

5.3 Add 0.5 mL of spiking solution to a 10 mL volumetric flask. Bring it to volume with the liquid portion of a sample or the extract of a solid sample..

5.4 Treat the sample as a normal analytical sample.

6.0 Control Charts



6.1 After samples are calculated, plot the following items on control charts:

6.1.1 Percent recovery of analytes in laboratory control samples.

6.1.2 Percent recovery for matrix spikes and matrix spike duplicates.

6.1.3 Initial calibration verification and continuing calibration verifications. Note: These are plotted on the same chart.



**Appendix B-2 – METHOD 8000A: Gas Chromatography**



**Table of Contents – Appendix B-2**

<b>Documents</b>
<b>METHOD 8000A: Gas Chromatography</b>



## METHOD 8000A

### GAS CHROMATOGRAPHY

#### 1.0 SCOPE AND APPLICATION

1.1 Gas chromatography is a quantitative technique useful for the analysis of organic compounds capable of being volatilized without being decomposed or chemically rearranged. Gas chromatography (GC), also known as vapor phase chromatography (VPC), has two subcategories distinguished by: gas-solid chromatography (GSC), and gas-liquid chromatography (GLC) or gas-liquid partition chromatography (GLPC). This last group is the most commonly used, distinguished by type of column adsorbent or packing.

1.2 The chromatographic methods are recommended for use only by, or under the close supervision of, experienced residue analysts.

#### 2.0 SUMMARY OF METHOD

2.1 Each organic analytical method that follows provides a recommended technique for extraction, cleanup, and occasionally, derivatization of the samples to be analyzed. Before the prepared sample is introduced into the GC, a procedure for standardization must be followed to determine the recovery and the limits of detection for the analytes of interest. Following sample introduction into the GC, analysis proceeds with a comparison of sample values with standard values. Quantitative analysis is achieved through integration of peak area or measurement of peak height.

#### 3.0 INTERFERENCES

3.1 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe or purging device must be rinsed out between samples with water or solvent. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of a solvent blank or of water to check for cross contamination. For volatile samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high organohalide concentrations, it may be necessary to wash out the syringe or purging device with a detergent solution, rinse it with distilled water, and then dry it in a 105°C oven between analyses.

#### 4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injections and all required accessories, including detectors, column supplies, recorder, gases, and syringes. A data system for measuring peak height and/or peak areas is recommended.

4.2 Gas chromatographic columns - See the specific determinative method. Other packed or capillary (open-tubular) columns may be used if the requirements



of Section 8.6 are met.

## 5.0 REAGENTS

5.1 See the specific determinative method for the reagents needed.

## 6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Section 4.1.

## 7.0 PROCEDURE

7.1 Extraction - Adhere to those procedures specified in the referring determinative method.

7.2 Cleanup and separation - Adhere to those procedures specified in the referring determinative method.

7.3 The recommended gas chromatographic columns and operating conditions for the instrument are specified in the referring determinative method.

### 7.4 Calibration

7.4.1 Establish gas chromatographic operating parameters equivalent to those indicated in Section 7.0 of the determinative method of interest. Prepare calibration standards using the procedures indicated in Section 5.0 of the determinative method of interest. Calibrate the chromatographic system using either the external standard technique (Section 7.4.2) or the internal standard technique (Section 7.4.3).

#### 7.4.2 External standard calibration procedure

7.4.2.1 For each analyte of interest, prepare calibration standards at a minimum of five concentrations by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.2.2 Inject each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph (e.g. 2-5  $\mu\text{L}$  injections, purge-and-trap, etc.). Tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each analyte. Alternatively, for samples that are introduced into the gas chromatograph using a syringe, the ratio of the response to the amount injected, defined as the calibration factor (CF), can be calculated for each analyte at each standard concentration. If the



percent relative standard deviation (%RSD) of the calibration factor is less than 20% over the working range, linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

$$\text{Calibration factor} = \frac{\text{Total Area of Peak}^*}{\text{Mass injected (in nanograms)}}$$

\* For multiresponse pesticides/PCBs, use the total area of all peaks used for quantitation.

7.4.2.3 The working calibration curve or calibration factor must be verified on each working day by the injection of one or more calibration standards. The frequency of verification is dependent on the detector. Detectors, such as the electron capture detector, that operate in the sub-nanogram range are more susceptible to changes in detector response caused by GC column and sample effects. Therefore, more frequent verification of calibration is necessary. The flame ionization detector is much less sensitive and requires less frequent verification. If the response for any analyte varies from the predicted response by more than  $\pm 15\%$ , a new calibration curve must be prepared for that analyte. For methods 8010, 8020, and 8030, see Table 3 in each method for calibration and quality control acceptance criteria.

$$\text{Percent Difference} = \frac{R_1 - R_2}{R_1} \times 100$$

where:

$R_1$  = Calibration Factor from first analysis.

$R_2$  = Calibration Factor from succeeding analyses.

#### 7.4.3 Internal standard calibration procedure

7.4.3.1 To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.4.3.2 Prepare calibration standards at a minimum of five concentrations for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards and dilute to volume with an appropriate solvent. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.



7.4.3.3 Inject each calibration standard using the same introduction technique that will be applied to the actual samples (e.g. 2 to 5  $\mu\text{L}$  injection, purge-and-trap, etc.). Tabulate the peak height or area responses against the concentration of each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$\text{RF} = (A_s C_{is}) / (A_{is} C_s)$$

where:

$A_s$  = Response for the analyte to be measured.

$A_{is}$  = Response for the internal standard.

$C_{is}$  = Concentration of the internal standard,  $\mu\text{g/L}$ .

$C_s$  = Concentration of the analyte to be measured,  $\mu\text{g/L}$ .

If the RF value over the working range is constant ( $< 20\%$  RSD), the RF can be assumed to be invariant, and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios,  $A_s/A_{is}$  versus RF.

7.4.3.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. The frequency of verification is dependent on the detector. Detectors, such as the electron capture detector, that operate in the sub-nanogram range are more susceptible to changes in detector response caused by GC column and sample effects. Therefore, more frequent verification of calibration is necessary. The flame ionization detector is much less sensitive and requires less frequent verification. If the response for any analyte varies from the predicted response by more than  $\pm 15\%$ , a new calibration curve must be prepared for that compound. For methods 8010, 8020, and 8030, see Table 3 in each method for calibration and quality control acceptance criteria.

## 7.5 Retention time windows

7.5.1 Before establishing windows, make sure the GC system is within optimum operating conditions. Make three injections of all single component standard mixtures and multiresponse products (i.e. PCBs) throughout the course of a 72 hour period. Serial injections over less than a 72 hour period result in retention time windows that are too tight.

7.5.2 Calculate the standard deviation of the three retention times (use any function of retention time; including absolute retention time, or relative retention time) for each single component standard. For multiresponse products, choose one major peak from the envelope and calculate the standard deviation of the three retention times for that peak. The peak chosen should be fairly immune to losses due to degradation and weathering in samples.



7.5.2.1 Plus or minus three times the standard deviation of the retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. For multiresponse analytes (i.e. PCBs), the analyst should use the retention time window, but should primarily rely on pattern recognition.

7.5.2.2 In those cases where the standard deviation for a particular standard is zero, the laboratory must substitute the standard deviation of a close eluting, similar compound to develop a valid retention time window.

7.5.3 The laboratory must calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. The data must be retained by the laboratory.

## 7.6 Gas chromatographic analysis

7.6.1 Introduction of organic compounds into the gas chromatograph varies depending on the volatility of the compound. Volatile organics are primarily introduced by purge-and-trap (Method 5030). However, there are limited applications (in Method 5030) where direct injection is acceptable. Use of Method 3810 or 3820 as a screening technique for volatile organic analysis may be valuable with some sample matrices to prevent overloading and contamination of the GC systems. Semivolatile organics are introduced by direct injection.

7.6.2 The appropriate detector(s) is given in the specific method.

7.6.3 Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with multi-concentration calibration standards. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded.

7.6.4 Direct Injection - Inject 2-5  $\mu\text{L}$  of the sample extract using the solvent flush technique, if the extract is manually injected. Smaller volumes (1.0  $\mu\text{L}$ ) can be injected, and the solvent flush technique is not required, if automatic devices are employed. Record the volume injected to the nearest 0.05  $\mu\text{L}$  and the resulting peak size in area units or peak height.

7.6.5 If the responses exceed the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, are acceptable if linearity is demonstrated. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

7.6.6 If peak detection is prevented by the presence of interferences, further cleanup is required.



7.6.7 Examples of chromatograms for the compounds of interest are frequently available in the referring analytical method.

7.6.8 Calibrate the system immediately prior to conducting any analyses (see Section 7.4). A mid-concentration standard must also be injected at intervals specified in the method and at the end of the analysis sequence. The calibration factor for each analyte to be quantitated, must not exceed a 15% difference when compared to the initial standard of the analysis sequence. When this criterion is exceeded, inspect the GC system to determine the cause and perform whatever maintenance is necessary (see Section 7.7) before recalibrating and proceeding with sample analysis. All samples that were injected after the standard exceeding the criterion must be reinjected to avoid errors in quantitation, if the initial analysis indicated the presence of the specific target analytes that exceeded the criterion.

7.6.9 Establish daily retention time windows for each analyte. Use the retention time for each analyte from Section 7.6.8 as the midpoint of the window for that day. The daily retention time window equals the midpoint  $\pm$  three times the standard deviation determined in Section 7.5.

7.6.9.1 Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Normally, confirmation is required: on a second GC column, by GC/MS if concentration permits, or by other recognized confirmation techniques. Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses.

7.6.9.2 Validation of GC system qualitative performance: Use the mid-concentration standards interspersed throughout the analysis sequence (Section 7.6.8) to evaluate this criterion. If any of the standards fall outside their daily retention time window, the system is out of control. Determine the cause of the problem and correct it (see Section 7.7). All samples that were injected after the standard exceeding the criteria must be reinjected to avoid false negatives and possibly false positives.

7.7 Suggested chromatography system maintenance - Corrective measures may require any one or more of the following remedial actions.

7.7.1 Packed columns - For instruments with injection port traps, replace the demister trap, clean, and deactivate the glass injection port insert or replace with a cleaned and deactivated insert. Inspect the injection end of the column and remove any foreign material (broken glass from the rim of the column or pieces of septa). Replace the glass wool with fresh deactivated glass wool. Also, it may be necessary to remove the first few millimeters of the packing material if any discoloration is noted, also swab out the inside walls of the column if any residue is noted. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body (described in Section 7.7.3) and/or repack/replace the column.



7.7.2 Capillary columns - Clean and deactivate the glass injection port insert or replace with a cleaned and deactivated insert. Break off the first few inches, up to one foot, of the injection port side of the column. Remove the column and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the column.

7.7.3 Metal injector body - Turn off the oven and remove the analytical column when the oven has cooled. Remove the glass injection port insert (instruments with off-column injection or Grob). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

7.7.3.1 Place a beaker beneath the injector port inside the GC oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene; catching the rinsate in the beaker.

7.7.3.2 Prepare a solution of deactivating agent (Sylon-CT or equivalent) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, serially rinse the injector body with toluene, methanol, acetone, and hexane. Reassemble the injector and replace the GC column.

## 7.8 Calculations

7.8.1 External standard calibration - The concentration of each analyte in the sample may be determined by calculating the amount of standard purged or injected, from the peak response, using the calibration curve or the calibration factor determined in Section 7.4.2. The concentration of a specific analyte is calculated as follows:

### Aqueous samples

$$\text{Concentration } (\mu\text{g/L}) = [(A_x)(A)(V_t)(D)] / [(A_s)(V_i)(V_s)]$$

where:

- $A_x$  = Response for the analyte in the sample, units may be in area counts or peak height.
- $A$  = Amount of standard injected or purged, ng.
- $A_s$  = Response for the external standard, units same as for  $A_x$ .
- $V_i$  = Volume of extract injected,  $\mu\text{L}$ . For purge-and-trap analysis,  $V_i$  is not applicable and therefore = 1.
- $D$  = Dilution factor, if dilution was made on the sample prior to analysis. If no dilution was made,  $D = 1$ , dimensionless.



$V_t$  = Volume of total extract,  $\mu\text{L}$ . For purge-and-trap analysis,  $V_t$  is not applicable and therefore = 1.

$V_s$  = Volume of sample extracted or purged, mL.

#### Nonaqueous samples

$$\text{Concentration } (\mu\text{g/kg}) = [(A_x)(A)(V_t)(D)]/[(A_s)(V_i)(W)]$$

where:

$W$  = Weight of sample extracted or purged, g. The wet weight or dry weight may be used, depending upon the specific applications of the data.

$A_x$ ,  $A_s$ ,  $A$ ,  $V_t$ ,  $D$ , and  $V_i$  have the same definition as for aqueous samples when a solid sample is purged (e.g., low concentration soil) for volatile organic analysis or for semivolatile organic and pesticide extracts. When the nonaqueous sample is extracted for purge and trap analysis,  $V_i$  = volume of methanol extract added to reagent water for purge and trap analysis.

7.8.2 Internal standard calibration - For each analyte of interest, the concentration of that analyte in the sample is calculated as follows:

#### Aqueous samples

$$\text{Concentration } (\mu\text{g/L}) = [(A_x)(C_{is})(D)]/[(A_{is})(RF)(V_s)]$$

where:

$A_x$  = Response of the analyte being measured, units may be in area counts or peak height.

$C_{is}$  = Amount of internal standard added to extract or volume purged, ng.

$D$  = Dilution factor, if a dilution was made on the sample prior to analysis. If no dilution was made,  $D = 1$ , dimensionless.

$A_{is}$  = Response of the internal standard, units same as  $A_x$ .

$RF$  = Response factor for analyte, as determined in Section 7.4.3.3.

$V_s$  = Volume of water extracted or purged, mL.

#### Nonaqueous samples

$$\text{Concentration } (\mu\text{g/kg}) = [(A_s)(C_{is})(D)]/[(A_{is})(RF)(W_s)]$$



where:

$W_s$  = Weight of sample extracted, g. Either a dry weight or wet weight may be used, depending upon the specific application of the data.

$A_s$ ,  $C_{is}$ ,  $D$ ,  $A_{is}$ , and  $RF$  have the same definition as for aqueous samples.

## 8.0 QUALITY CONTROL

8.1 Each laboratory that uses these methods is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document quality data. The laboratory should maintain records to document the quality of the data generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard should be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.2 Before processing any samples, the analyst should demonstrate, through the analysis of a reagent blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is extracted or there is a change in reagents, an organic-free reagent water blank should be processed as a safeguard against chronic laboratory contamination. The blank samples should be carried through all stages of the sample preparation and measurement steps.

8.3 For each analytical batch (up to 20 samples), a reagent blank, matrix spike, and duplicate or matrix spike duplicate should be analyzed (the frequency of the spikes may be different for different monitoring programs). The blank and spiked samples should be carried through all stages of the sample preparation and measurement steps.

8.4 The experience of the analyst performing gas chromatography is invaluable to the success of the methods. Each day that analysis is performed, the daily calibration sample should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal?; Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still good, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g. column changed), recalibration of the system should take place.

### 8.5 Required instrument QC

8.5.1 Step 7.4 requires that the %RSD vary by < 20% when comparing calibration factors to determine if a five point calibration curve is linear.



8.5.2 Section 7.4 sets a limit of  $\pm 15\%$  difference when comparing daily response of a given analyte versus the initial response. For Methods 8010, 8020, and 8030, follow the guidance on limits specified in Section 7.4.3.4. If the limit is exceeded, a new standard curve should be prepared unless instrument maintenance corrects the problem for that particular analyte.

8.5.3 Step 7.5 requires the establishment of retention time windows.

8.5.4 Section 7.6.8 sets a limit of  $\pm 15\%$  difference when comparing the response from the continuing calibration standard of a given analyte versus any succeeding standards analyzed during an analysis sequence.

8.5.5 Step 7.6.9.2 requires that all succeeding standards in an analysis sequence should fall within the daily retention time window established by the first standard of the sequence.

8.6 To establish the ability to generate acceptable accuracy and precision, the analyst should perform the following operations.

8.6.1 A quality control (QC) check sample concentrate is required containing each analyte of interest. The QC check sample concentrate may be prepared from pure standard materials, or purchased as certified solutions. If prepared by the laboratory, the QC check sample concentrate should be made using stock standards prepared independently from those used for calibration.

8.6.1.1 The concentration of the QC check sample concentrate is highly dependent upon the analytes being investigated. Therefore, refer to Method 3500, Section 8.0 for the required concentration of the QC check sample concentrate.

8.6.2 Preparation of QC check samples

8.6.2.1 Volatile organic analytes (Methods 8010, 8020, and 8030) - The QC check sample is prepared by adding 200  $\mu\text{L}$  of the QC check sample concentrate (Step 8.6.1) to 100 mL of water.

8.6.2.2 Semivolatile organic analytes (Methods 8040, 8060, 8070, 8080, 8090, 8100, 8110, and 8120) - The QC check sample is prepared by adding 1.0 mL of the QC check sample concentrate (Step 8.6.1) to each of four 1-L aliquots of water.

8.6.3 Four aliquots of the well-mixed QC check sample are analyzed by the same procedures used to analyze actual samples (Section 7.0 of each of the methods). For volatile organics, the preparation/analysis process is purge-and-trap/gas chromatography. For semivolatile organics, the QC check samples should undergo solvent extraction (see Method 3500) prior to chromatographic analysis.

8.6.4 Calculate the average recovery ( $\bar{x}$ ) in  $\mu\text{g/L}$ , and the standard deviation of the recovery ( $s$ ) in  $\mu\text{g/L}$ , for each analyte of interest using the four results.



8.6.5 For each analyte compare  $s$  and  $\bar{x}$  with the corresponding acceptance criteria for precision and accuracy, respectively, given the QC Acceptance Criteria Table at the end of each of the determinative methods. If  $s$  and  $\bar{x}$  for all analytes of interest meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual  $s$  exceeds the precision limit or any individual  $\bar{x}$  falls outside the range for accuracy, then the system performance is unacceptable for that analyte.

NOTE: The large number of analytes in each of the QC Acceptance Criteria Tables present a substantial probability that one or more will fail at least one of the acceptance criteria when all analytes of a given method are determined.

8.6.6 When one or more of the analytes tested fail at least one of the acceptance criteria, the analyst should proceed according to Step 8.6.6.1 or 8.6.6.2.

8.6.6.1 Locate and correct the source of the problem and repeat the test for all analytes of interest beginning with Step 8.6.2.

8.6.6.2 Beginning with Step 8.6.2, repeat the test only for those analytes that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Step 8.6.2.

8.7 The laboratory should, on an ongoing basis, analyze a reagent blank and a matrix spiked duplicate for each analytical batch (up to a maximum of 20 samples/batch) to assess accuracy. For soil and waste samples where detectable amounts of organics are present, replicate samples may be appropriate in place of spiked duplicates. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

8.7.1 The concentration of the spike in the sample should be determined as follows:

8.7.1.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the spike should be at that limit, or 1 to 5 times higher than the background concentration determined in Step 8.7.2, whichever concentration would be larger.

8.7.1.2 If the concentration of a specific analyte in a water sample is not being checked against a limit specific to that analyte, the spike should be at the same concentration as the QC reference sample (Step 8.6.2) or 1 to 5 times higher than the background concentration determined in Step 8.7.2, whichever concentration would be larger. For other matrices, the recommended spiking concentration is 20 times the EQL.



8.7.1.3 For semivolatile organics, it may not be possible to determine the background concentration levels prior to spiking (e.g. maximum holding times will be exceeded). If this is the case, the spike concentration should be (1) the regulatory concentration limit, if any; or, if none (2) the larger of either 5 times higher than the expected background concentration or the QC reference sample concentration (Step 8.6.2). For other matrices, the recommended spiking concentration is 20 times the EQL.

8.7.2 Analyze one unspiked and one spiked sample aliquot to determine percent recovery of each of the spiked compounds.

8.7.2.1 Volatile organics - Analyze one 5-mL sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a new QC reference sample concentrate (Step 8.6.1) appropriate for the background concentration in the sample. Spike a second 5-mL sample aliquot with 10  $\mu$ L of the QC reference sample concentrate and analyze it to determine the concentration after spiking (A) of each analyte. Calculate each percent recovery (p) as  $100(A - B)/T$ , where T is the known true value of the spike.

8.7.2.2 Semivolatile organics - Analyze one sample aliquot (extract of 1-L sample) to determine the background concentration (B) of each analyte. If necessary, prepare a new QC reference sample concentrate (Step 8.6.1) appropriate for the background concentration in the sample. Spike a second 1-L sample aliquot with 1.0 mL of the QC reference sample concentrate and analyze it to determine the concentration after spiking (A) of each analyte. Calculate each percent recovery (p) as  $100(A - B)/T$ , where T is the known true value of the spike.

8.7.3 Compare the percent recovery (p) for each analyte in a water sample with the corresponding criteria presented in the QC Acceptance Criteria Table found at the end of each of the determinative methods. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1. If spiking was performed at a concentration lower than the QC reference sample concentration (Step 8.6.2), the analyst should use either the QC acceptance criteria presented in the Tables, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of an analyte: (1) Calculate accuracy ( $x'$ ) using the equation found in the Method Accuracy and Precision as a Function of Concentration Table (appears at the end of each determinative method), substituting the spike concentration (T) for C; (2) calculate overall precision ( $S'$ ) using the equation in the same Table, substituting  $x'$  for x; (3) calculate the range for recovery at the spike concentration as  $(100x'/T) \pm 2.44(100S'/T)\%$ .

8.7.4 If any individual p falls outside the designated range for recovery, that analyte has failed the acceptance criteria. A check standard containing each analyte that failed the criteria should be



analyzed as described in Step 8.8.

8.8 If any analyte in a water sample fails the acceptance criteria for recovery in Step 8.7, a QC reference standard containing each analyte that failed should be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC reference standard will depend upon the number of analytes being simultaneously tested, the complexity of the sample matrix, and the performance of the laboratory. If the entire list of analytes given in a method should be measured in the sample in Step 8.7, the probability that the analysis of a QC check standard will be required is high. In this case, the QC check standard should be routinely analyzed with the spiked sample.

8.8.1 Preparation of the QC check sample - For volatile organics, add 10  $\mu$ L of the QC check sample concentrate (Step 8.6.1 or 8.7.2) to 5 mL of water. For semivolatile organics, add 1.0 mL of the QC check sample concentrate (Step 8.6.1 or 8.7.2) to 1 L of water. The QC check sample needs only to contain the analytes that failed criteria in the test in Step 8.7. Prepare the QC check sample for analysis following the guidelines given in Method 3500 (e.g. purge-and-trap, extraction, etc.).

8.8.2 Analyze the QC check sample to determine the concentration measured (A) of each analyte. Calculate each percent recovery ( $p_s$ ), as  $100(A/T)\%$ , where T is the true value of the standard concentration.

8.8.3 Compare the percent recovery ( $p_s$ ) for each analyte with the corresponding QC acceptance criteria found in the appropriate Table in each of the methods. Only analytes that failed the test in Step 8.7 need to be compared with these criteria. If the recovery of any such analyte falls outside the designated range, the laboratory performance for that analyte is judged to be out of control, and the problem should be immediately identified and corrected. The result for that analyte in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.9 As part of the QC program for the laboratory, method accuracy for each matrix studied should be assessed and records should be maintained. After the analysis of five spiked samples (of the same matrix type) as in Step 8.7, calculate the average percent recovery ( $\bar{p}$ ) and the standard deviation of the percent recovery ( $s_p$ ). Express the accuracy assessment as a percent recovery interval from  $\bar{p} - 2s_p$  to  $\bar{p} + 2s_p$ . If  $\bar{p} = 90\%$  and  $s_p = 10\%$ , for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment for each analyte on a regular basis (e.g. after each five to ten new accuracy measurements).

8.10 Calculate surrogate control limits as follows:

8.10.1 For each sample analyzed, calculate the percent recovery of each surrogate in the sample.

8.10.2 Calculate the average percent recovery ( $\bar{p}$ ) and standard deviation of the percent recovery ( $s$ ) for each of the surrogates when



surrogate data from 25 to 30 samples for each matrix is available.

8.10.3 For a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard. This should be done as follows:

$$\text{Upper Control Limit (UCL)} = p + 3s$$

$$\text{Lower Control Limit (LCL)} = p - 3s$$

8.10.4 For aqueous and soil matrices, these laboratory established surrogate control limits should, if applicable, be compared with the control limits in Tables A and B of Methods 8240 and 8270, respectively. The limits given in these methods are multi-laboratory performance based limits for soil and aqueous samples, and therefore, the single-laboratory limits established in Step 8.10.3 should fall within those given in Tables A and B for these matrices.

8.10.5 If recovery is not within limits, the following is required.

- Check to be sure there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration."

8.10.6 At a minimum, each laboratory should update surrogate recovery limits on a matrix-by-matrix basis, annually.

8.11 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer should be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

## 9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in the referring analytical methods were obtained using water. Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.



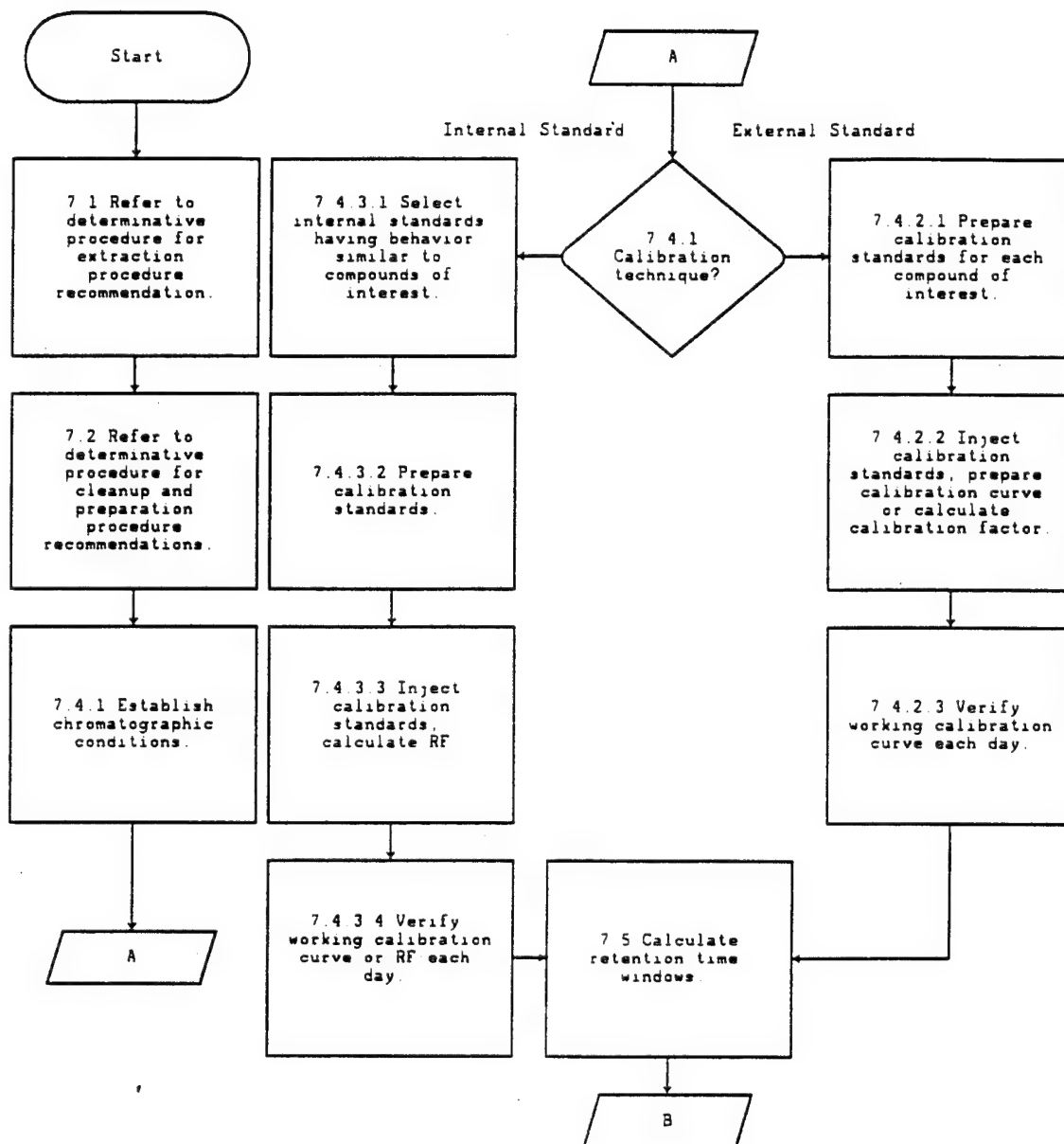
9.2 Refer to the determinative method for specific method performance information.

## 10.0 REFERENCES

1. U.S. EPA 40 CFR Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," October 26, 1984.
2. U.S. EPA Contract Laboratory Program, Statement of Work for Organic Analysis, July 1985, Revision.

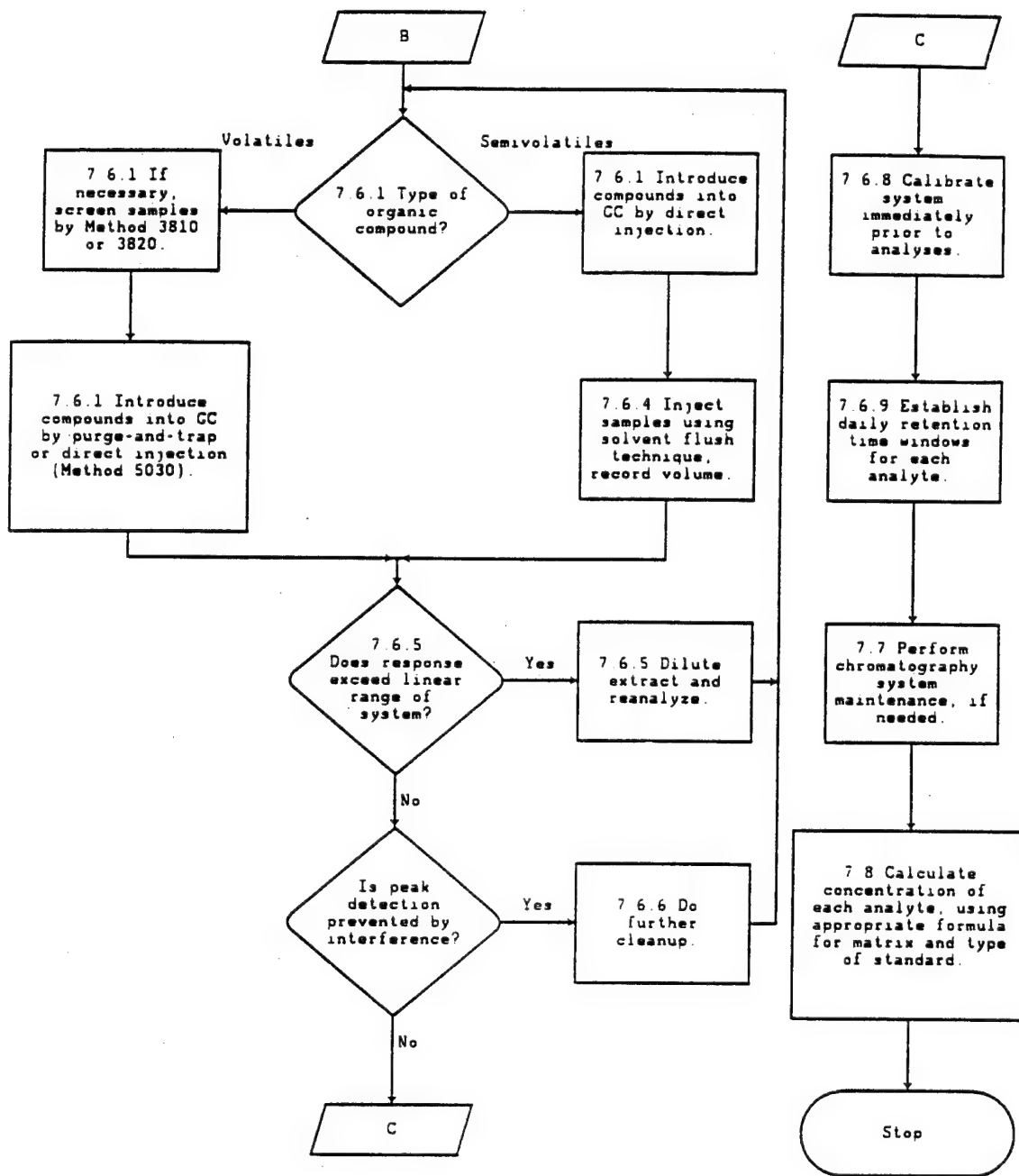


# METHOD 8000A GAS CHROMATOGRAPHY





METHOD 8000A  
continued





### **Appendix B-3 – Procedures for YSI 600 Sonde**



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# 1. INTRODUCTION

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## 1.1 DESCRIPTION

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The 600XL Environmental Monitoring System is a multiparameter, water quality measurement, and data collection system. It is intended for use in research, assessment, and regulatory compliance applications.

Measurement parameters include:

- Dissolved Oxygen
- Conductivity
- Specific Conductance
- Salinity
- Total Dissolved Solids
- Resistivity
- Temperature
- pH
- ORP
- Depth
- Level

The YSI Model 600XL is similar in appearance and performance to the original YSI Model 600 series, but is characterized by three significant enhancements. First, the Model 600XL offers field replaceable sensors. Second, the instrument can be configured with a factory-installed depth sensor module. Third, the unit is now available with an ORP sensor. Like the original Model 600, the 600XL has significant similarities to the YSI Model 6000 series, but also differs from that larger instrument in several ways. First, the Model 600XL does not have internal battery capability, and therefore must be powered from an external power source such as an AC adapter, battery pack, or terminal device. Second, the Model 600XL has no internal logging capability, and therefore the Model 600XL must be used with a terminal, data logger, data collection platform, or computer. Finally, several sensors, such as turbidity, nitrate, and ammonium, which are available on the Model 6000, cannot be used with the 600XL.

The Model 600XL is ideal for profiling and monitoring water conditions in industrial and waste water effluents, lakes, rivers, wetlands, estuaries, coastal waters, and monitoring wells. It can be left unattended for weeks at a time with measurement parameters sampled at your setup interval and data transmitted to your computer or logging device. The Model 600XL can be used 200 feet below the water's surface or in as little as a few inches of water. The fast sensor response of the Model 600XL makes it ideal for vertical profiling. Its very small size allows it to fit down 2 inch diameter monitoring wells.

The Model 600XL is equipped with YSI's patented Rapid Pulse Dissolved Oxygen Sensor which exhibits low stirring dependence, and therefore provides accurate results without an expensive and bulky stirrer. Because stirring is not required, external battery life is extended. In addition, because of the nature of the technology, sensor drift caused by passive fouling is minimized.



The Model 600XL communicates with an ASCII terminal or a computer with a terminal emulation program. Use of the 600XL with our 610 D and 610 DM display/loggers provides an ideal system for profiling or spot sampling.

Every Model 600XL comes with IBM-compatible PC based software for simple and convenient setup and data handling. Reports and plots are automatically generated and their presentation easily customized. Data is easily exported to any spreadsheet program for more sophisticated data processing.

The RS-232C and SDI-12 interfaces provide maximum versatility for system networking and real time data collection. Several Model 600XL units are easily installed as a network, providing valuable water quality data at a variety of locations. For real time results, the Model 600XL can interface to radio telemetry systems, satellite, modem and cellular phone data collection platforms.

The Model 600XL is available with an economical built-in cable of various lengths, or with a sonde-mounted connector. Optional interface cables in several lengths are available for interfacing with a computer or terminal. These cables are waterproof at the sonde end and can be used in the lab or field.

See Appendix D for a complete list of accessories and calibration reagents.

## **1.2 GENERAL SPECIFICATIONS**

---

See also Section 1.3 Sensor Specifications.

### **Operating Environment**

Medium: fresh, sea, or polluted water

Temperature: -5 to +45 °C

Depth: 0 to 200 (61 meters)

**Storage Temperature:** -40 to +60 °C

**Material:** PVC, Stainless Steel

### **Dimensions and weight with a 50 foot integral cable.**

Diameter: 1.6 inches (4.06 cm)

Length: 14 inches (35.56 cm)

Weight: 4.9 pounds (2.22 kg)

### **Dimensions with depth sensor bulkhead installed and no attached cable.**

Diameter: 1.6 inches (4.06 cm)

Length: 20.75 inches (52.7 cm)

Weight: 1.75 pounds (0.8 kg)



### **Computer Interface**

RS-232C

SDI-12

### **Software**

IBM PC compatible computer, 3 1/2 or 5 1/4 inch, high or low density floppy disk drive.

Minimum RAM requirement: 256 K bytes

Optional graphic adapter for plotting

### **Power**

External 12 VDC (8 to 13.8 VDC)

## **1.3 SENSOR SPECIFICATIONS**

---

The following are typical performance specifications for each sensor.

### **Depth - Medium**

Sensor Type.....Stainless steel strain gauge

Range.....0 to 200 ft (61 m)

Accuracy.....+/- 0.4 ft (0.12 m)

Resolution.....0.001 ft (0.001 m)

### **Depth - Shallow**

Sensor Type.....Stainless steel strain gauge

Range.....0-30 ft (9.1 m)

Accuracy ..... +/- 0.06 ft (0.018 m)

Range.....0.001 ft (0.001 m)

### **Temperature**

Sensor Type.....Thermistor

Range.....-5 to 45 °C

Accuracy.....+/- 0.15 °C (optional configuration at +/- 0.05 °C)

Resolution.....0.01 °C

### **Dissolved Oxygen, % saturation**

Sensor Type.....Rapid Pulse - Clark type, polarographic

Range.....0 to 200 % air saturation

Accuracy.....+/- 2 % air saturation

Resolution.....0.1 % air saturation



**Dissolved Oxygen, mg/L** (Calculated from % air saturation, temperature and salinity)

Sensor Type..... Rapid Pulse - Clark type polarographic

Range.....0 to 20 mg/L

Accuracy.....+/- 0.2 mg/L

Resolution.....0.01 mg/L

**Conductivity \***

Sensor Type.....4 electrode cell

Range.....0 to 100 mS/cm

Accuracy.....+/- 0.5% of reading + 0.001 mS/cm

Resolution.....0.01 mS/cm or 1 uS/cm

**Salinity**

Sensor Type.....Calculated from conductivity and temperature

Range.....0 to 70 ppt

Accuracy.....+/- 1.0% of reading or 0.1 ppt, whichever is greater

Resolution.....0.01 ppt

**pH**

Sensor Type.....Glass combination electrode

Range.....2 to 14 units

Accuracy.....+/- 0.2 units

Resolution.....0.01 units

**pH - Low Ionic Strength**

Sensor Type.....Glass combination electrode with open junction and low impedance glass

Range.....2 to 14 units

Accuracy.....+/- 0.2 units

Resolution.....0.01 units

**ORP**

Sensor type..... Platinum ring

Range.....-999 to 999 mv

Accuracy.....+/-20 mv

Resolution.....0.1 mv

---

\* Report outputs of specific conductance (conductivity corrected to 25 C), resistivity, and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (Ed 1989).



## **1.4 HOW TO USE THIS MANUAL**

---

This manual provides information for operating and maintaining the Model 600XL Environmental Monitoring System. Sections 1 through 3 provide an overview of setup, calibration, and operational procedures. These first three chapters should provide enough information for you to understand the basic capabilities of the 600XL system and begin sampling. Sections 4 through 9 provide a more detailed explanation of system operations, software, principles of operation, maintenance, and performance troubleshooting. Appendices A-G provide information about health and safety, warranty, accessories, and options.

**NOTE:** Because of the many features and applications of this versatile product, some sections of this manual may not apply to the specific system you have purchased.

This manual is organized to let you quickly understand and operate the 600XL system. However, it cannot be stressed too strongly that informed and safe operation is more than just knowing which buttons to push. An understanding of the principles of operation, calibration techniques, and system setup is necessary to obtain accurate and meaningful results.

Regular maintenance is required to keep the 600XL functioning properly. Precautions regarding the handling of reagents are also essential for the safety of system operators (see Appendix A for health and safety information).

The early parts of this manual will teach you how to get the 600XL system running. Additional topics are included to help you understand the science it employs, how to use it most effectively and safely, and how to keep it operating correctly.

The 600XL can be purchased with external battery or power supply capability. Additionally, all probes, cables and accessories can be ordered as options or ordered together as a system.

If you have any questions about this product or its application, please contact our customer service department or authorized dealer for assistance. See Appendix C for contact information.



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## 2. INITIAL SETUP

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### 2.1 UNPACKING

---

Remove the instrument from the shipping container. Be careful not to discard any parts or supplies. Check off all items on the packing list and inspect all assemblies and components for damage. If any parts are damaged or missing, contact your representative immediately. If you do not know from which dealer your 600XL was purchased, refer to Appendix C for contact information.

**NOTE:** Reagents for the 600XL are not packaged in the same carton as the instrument. These materials must be ordered separately and will arrive in a separate package.

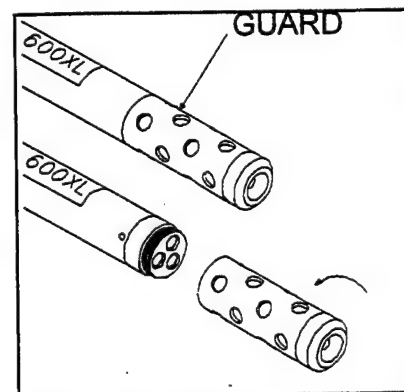
### 2.2 SONDE SETUP

---

#### SENSORS

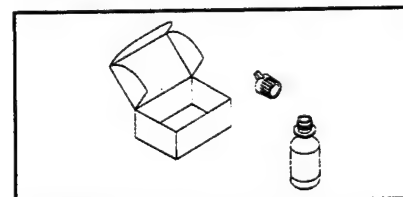
---

1. Remove the Model 600XL probe guard by hand.

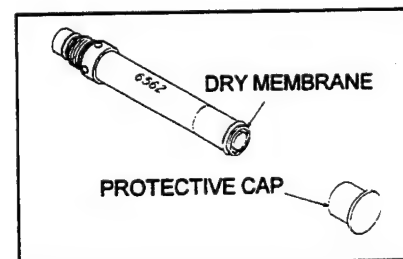


2. **NOTE:** Step 2 is for the preparation of the 6562 dissolved oxygen probe only. To install other probes, proceed to step 3.

A. Open the membrane kit and prepare electrolyte. Dissolve the KCl in the dropper bottle by filling it to the neck with distilled water and shaking until the solid is fully dissolved. After dissolution is complete, wait 10-15 minutes until the solution is free of bubbles.



- B. Remove protective cap and the dry membrane from the 600XL dissolved oxygen probe. **NOTE:** The dissolved oxygen probe is shipped with a protective dry membrane on the sensor tip. It is very important not to scratch or contaminate the sensor tip. Handle the new probe with care. Avoid touching or hitting of the sensor tip.





C. Hold the probe in a vertical position and apply a few drops of KCl solution to the tip. The fluid should completely fill the small moat around the electrodes and form a meniscus on the tip of the sensor. Be sure no air bubbles are stuck to the face of the sensor. If necessary, shake off the electrolyte and start over.

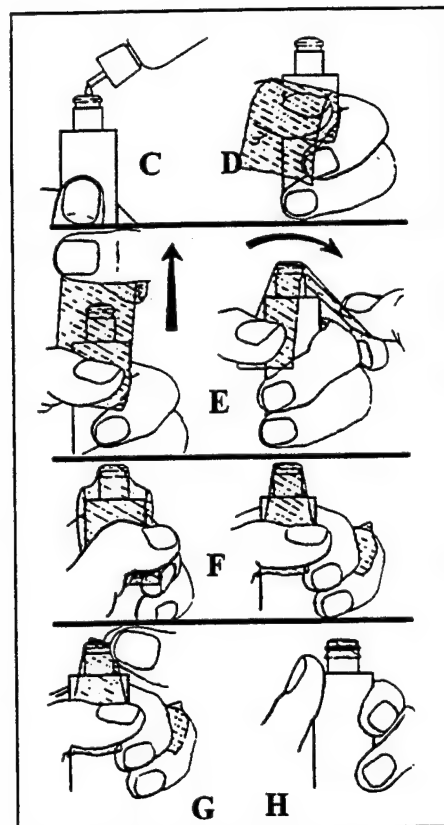
D. Secure a membrane between your left thumb and the probe body. Always handle the membrane with care, touching it at the ends only.

E. With the thumb and forefinger of your right hand, grasp the free end of the membrane. With one continuous motion, gently stretch it up, over, and down the other side of the sensor. The membrane should conform to the face of the sensor.

F. Secure the end of the membrane under the forefinger of your left hand.

G. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface with your fingers. There should be no wrinkles or trapped air bubbles. Small wrinkles may be removed by lightly tugging on the edges of the membrane. If bubbles are present, remove the membrane and repeat steps C-G.

H. Trim off any excess membrane with a sharp knife or scissors. Make sure the temperature sensor is not covered by excess membrane. Being careful not to get water in the connector, rinse off the excess KCl solution.

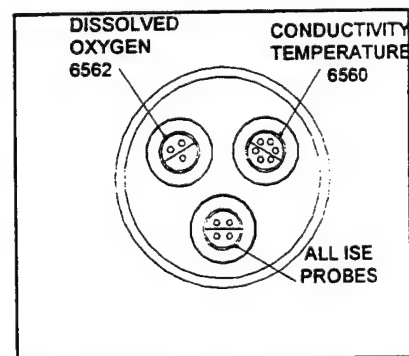
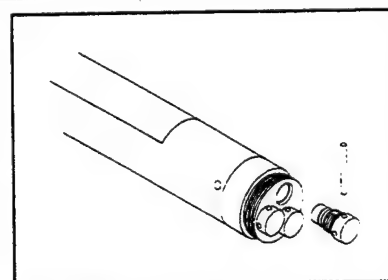


NOTE: Some users find it more convenient to mount the sensor vertically in a vise with rubber jaws while applying the electrolyte and membrane.

3. Using the probe installation tool supplied in the 6570 maintenance kit, remove the port plugs and locate the port with the connector corresponding to the probe you wish to install.

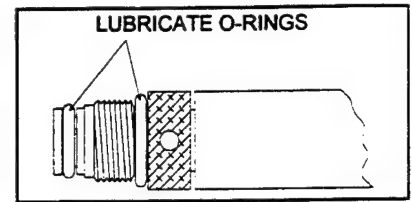
Note:

- 6562 Dissolved oxygen probe = 3-pin connector
- 6560 Conductivity/Temperature = 6-pin connector
- 6561 pH probe = 4 pin connector
- 6563 ORP probe = 4 pin connector
- 6565 Combination pH/ORP probe = 4 pin connector
- 6564 LIS pH probe = 4 pin connector
- 6567 combination LIS pH/ORP probe = 4 pin connector



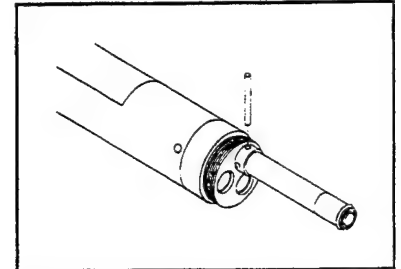


4. Apply a thin coat of O-ring lubricant (supplied in the YSI 6570 maintenance kit) to the O-rings on the connector side of the probe.

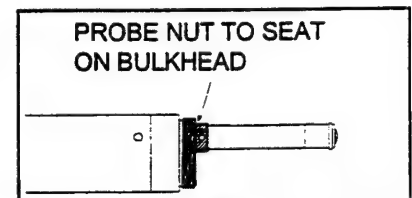


5. NOTE: Before installing probe into sonde, be sure probe port is free of moisture.

Insert the probe into the correct port and gently rotate the probe until the two connectors align.

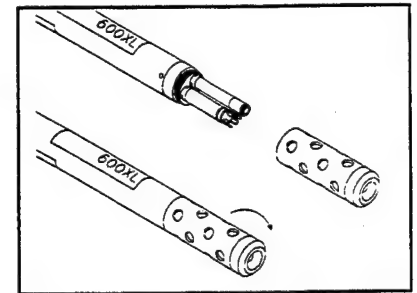


6. With connectors aligned, screw down the probe nut using the probe installation tool. **CAUTION:** Use care not to cross thread the probe nut. Seat nut on face of bulkhead. Do not over tighten.



7..Repeat steps 3-6 for all remaining probes.

8. Replace the 600XL probe guard.



## CABLES

Some versions of the Model 600XL have permanently attached cables. If your 600XL has a cable which is non-detachable (no stainless steel connector), parts of this section will not be relevant.

To attach a cable to the 600XL, remove the waterproof cap from the sonde connector and set it aside carefully for later reassembly. Now connect your YSI PC interface cable to the sonde connector. A built-in "key" will ensure proper pin alignment; rotate the cable gently until the "key" engages and then tighten the connectors together by rotating clockwise.

The other end of the cable is a military-style 8-pin connector. This connector plugs directly into the 610 D and 610 DM display/loggers. Most other applications will require the use of an adapter. For example, to connect the 600XL to a computer, use a YSI 6095 MS8 to DB-9 adapter.



## POWER

---

Some type of external power supply is required to power the 600XL sonde. For laboratory setup and calibration with the sonde interfaced to a computer, the YSI 6038 (110 VAC) or 6037 (220 VAC) is ideal. Most adapters include a short pigtail for power that plugs into the power supply. After attaching the three pin connector on the power supply to the pigtail, simply plug the power supply into the appropriate outlet. If you have purchased a 610-series display/logger for use with your 600XL, attachment of the cable to the 610 will allow your sonde to be powered from the batteries in the display/logger or from the 610 power supply if its batteries are not fully charged.



### 3. BASIC OPERATION

---

In the previous Section, you learned how to install probes and set up the PC6000 and 600XL sonde software. In this Section, you will learn how to calibrate and run the Model 600XL and how to view your data on a computer display. If you choose to use your 600XL with a 610-series display/logger, refer to the operations manual for the 610 to obtain similar instructions to those provided below.

#### 3.1 CALIBRATION TIPS

---

**WARNING:** Reagents used to calibrate and check this instrument may be hazardous to your health. Refer to Appendix A for health and safety information.

Before you begin the calibration procedures outlined below, you may find it helpful to follow some or all of these calibration tips.

1. Remove the sonde stainless steel weight on the bottom of the sonde guard by turning it counterclockwise. This allows the calibration solutions access to the probes with minimal displacement of fluid within the calibration cup. Additionally, carry-over from one solution to the next is reduced.
2. Fill a large bucket with ambient temperature water for rinsing the sonde between calibration solutions.
3. Have several clean, absorbent paper towels or cotton cloths available to dry the sonde between rinses and calibration solutions. It is important to remove as much residual liquid as possible from the sonde after each rinse. Shake the sonde to remove excess rinse water from the inside of the guard. Then dry the outside of the sonde and guard. Drying the sonde and probes in this way reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration, particularly lower conductivity calibration standards.
4. It is not necessary to remove the probe guard to rinse and dry the probes between calibration solutions. The inaccuracy resulting from simply rinsing the probe compartment and drying the outside of the sonde is minimal.

#### 3.2 CALIBRATION PROCEDURES

---

**WARNING:** Calibration reagents may be hazardous to your health. Refer to Appendix A for health and safety information.

A calibration cup is supplied with the Model 600XL. Because the calibration cup fits over the outside of the sonde sensor guard, it is not recommended or necessary to remove the guard to calibrate the sensors. Follow the procedures below to calibrate the sensors. Only *basic* DO percent



saturation, Conductivity, pH, and Depth calibration procedures are discussed in this section. Temperature does not require calibration and is, therefore, not included in the Calibrate menu. ORP calibration is required only infrequently and is discussed in Section 4.2. For more detailed calibration procedures, which can be used to enhance the accuracy of some measurements, see Section 4.2.

From the sonde Main menu select **2. Calibrate**. The Calibrate menu will be displayed.

-----Calibrate-----	
1. Conductivity	4. ISE1-pH
2. Dissolved Oxy	5. ISE2-Orp
3. Pressure-Abs	
Select option (0 for previous menu):	

Selection of any of the parameters from the Calibrate menu listing will require the user to input a numerical value and then press Enter. For example, for calibration of specific conductance, the following display will be shown during the calibration sequence.

Enter SpCond in mS/cm (10):
-----------------------------

The number in parentheses is the default value of this parameter and will be used in the calibration if *only* Enter is pressed without typing in another value. Similar prompts will be displayed calibration of all parameters, but for some sensors, such as pH, no default values are provided. In these cases, the user must input a numerical value and then press Enter.

After the calibration value is input and Enter is pressed, a real-time display similar to the following will then appear on the screen. Note that all parameters which have been enabled will appear - not just the one being calibrated at the moment. The user should carefully observe the stabilization of the readings of the parameter which is being calibrated and, when the readings are stable for approximately 30 seconds, press Enter to implement the calibration.

Temp	SpCond	Cond	Sal
C	mS/cm	mS/cm	ppt
-----			
To calibrate, press <Enter> when the readings are stable.			
23.00	10.0	10.0	15.7
Select option (0 for previous menu)			



**NOTE:** If an ERROR message appears, begin the calibration procedure again. Be certain that the value you enter for the calibration standard is correct. Also see Section 8, Troubleshooting for more information on error messages.

**CAUTION:** Be certain to **immerse the entire sonde** in solution standards for calibration of all parameters. Most calibrations require readings not only from the sensor being calibrated but also from the temperature sensor.

Specific start-up calibration procedures for all sensors which commonly require calibration are provided in the following paragraphs of this section. Remember that these are basic protocols designed to get the user up and running with regard to the 600XL. The more-detailed discussion of sensor calibration found in Section 4.2 should be examined prior to use of the instrument in the field.

**NOTE:** If the particular sensor listed is not installed in your sonde, proceed to the next sensor until the calibration protocol is complete.

## **CONDUCTIVITY**

---

**NOTE:** This procedure calibrates not only conductivity, but also specific conductance, salinity, and total dissolved solids.

Place approximately 300 mL of conductivity standard in a clean and dry calibration cup. The conductivity standard you choose should be within the same conductivity range as the water you are preparing to sample. However, we do not recommend using standard less than 1 mS/cm. For example:

- For fresh water choose a 1 mS/cm conductivity standard.
- For brackish water choose a 10 mS/cm conductivity standard.
- For sea water choose a 50 mS/cm conductivity standard.

**Caution:** Before proceeding insure that the sensor is as dry as possible. Ideally, rinse the conductivity sensor with a small amount of standard that can be discarded. Be certain that you avoid cross contamination of standard solutions with other solutions. Make certain that there are no salt deposits around the oxygen and pH/ORP probes, particularly if you are employing standards of low conductivity.

Without removing the sonde guard, *carefully* immerse the probe end of the sonde into the solution. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell. The probe must be completely immersed past its vent hole.

Allow at least one minute for temperature equilibration before proceeding.



From the Calibrate menu, select **1. Conductivity** to access the Conductivity calibration procedure and then **1. SpCond.** to access the specific conductance calibration procedure. Enter the calibration value of the standard you are using (mS/cm at 25 C) and press Enter. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

Observe the readings under Specific Conductance or Conductivity and when they show no significant change for approximately 30 seconds, press Enter. The screen will indicate that the calibration has been accepted and prompt you to press Enter again to return to the Calibrate menu.

Rinse the sonde in tap or purified water and dry the sonde.

## **pH 2-POINT**

---

Place approximately 200 mL of pH 7 buffer in a clean calibration cup. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

From the Calibrate menu, select **4. ISE1 pH** to access the pH calibration choices and then **2. 2-Point**. Press Enter and input the value of the buffer (7 in this case) at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. The display will indicate that the calibration is accepted.

After the pH 7 calibration is complete, press Enter again, as instructed on the screen, to continue.

Rinse the sonde in water and dry the sonde before proceeding to next step.

Place approximately 200 mL of a second pH buffer solution in a clean calibration cup. The second buffer might be pH 4 if the sample is expected to be acidic or pH 10 if the sample is expected to be basic. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Press Enter and input the value of the second buffer at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. After the second value calibration is complete, press Enter again, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

Thoroughly rinse and dry the calibration cups for future use.



## DISSOLVED OXYGEN

---

Place approximately 1/8 inch of water or a wet sponge in the bottom of the calibration cup. Place the probe end of the sonde into the calibration cup. Make certain that the DO and the temperature probes are not immersed in the water. Wait approximately 10 minutes for the air in the calibration cup to become water saturated and for the temperatures of the thermistor and the oxygen probe to equilibrate. Make certain that the calibration cup is vented to the atmosphere.

From the **Calibrate** menu, select **2. Dissolved Oxy** to access the DO % calibration procedure.

Enter the current barometric pressure in mm of Hg. *Remember that barometer readings which appear in meteorological reports are generally corrected to sea level and are not useful for you calibration procedure unless they are uncorrected.*

**NOTE:** Inches of Hg x 25.4 mm/inch = mm Hg

Press **Enter** and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize. Observe the readings under DO % and when they show no significant change for approximately 30 seconds, press **Enter**. The screen will indicate that the calibration has been accepted and prompt you to press **Enter** again to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

**NOTE:** Calibration of dissolved oxygen in the DO % procedure also results in calibration of the DO mg/L mode and vice versa.

**NOTE:** The above procedure is designed to calibrate your dissolved oxygen sensor for use in sampling applications where the sensor is being pulsed continuously in the Run mode because both "Auto sleep" and "Wait for DO" functions have been disabled as described in Section 2. If your 600XL is to be used in a monitoring application in which data is being captured to a computer or data collection platform, "Auto sleep" and "Wait for DO" will be activated and the calibration displays will be somewhat different. See Section 4 for details.

## DEPTH

---

Following the DO calibration, leave the sonde in water-saturated air. Make certain that the sonde is not submerged in water for the depth calibration.

From the Calibrate menu, select **4. Depth** to access the depth calibration procedure. Input 0.00 or some known sensor offset in feet. Press **Enter** and monitor the stabilization of the depth readings with time. When no significant change occurs for approximately 30 seconds, press **Enter** to confirm the calibration and zero the sensor with regard to current barometric pressure.



After depth is zeroed, press **Enter** again, as instructed on the screen, to return to the Calibrate menu.

The sensors are now calibrated. Press **Esc** until the sonde Main menu is displayed.



## **Appendix B-4 – Procedures for YSI 6000 Sonde**



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# **1. INTRODUCTION**

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## **1.1 DESCRIPTION**

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The YSI Model 6000 Environmental Monitoring System is a multiparameter, water quality measurement, and data logging system. It is intended for use in research, assessment, and regulatory compliance applications.

Standard measurement parameters include:

- » Dissolved Oxygen
- » Conductivity
- » Specific Conductance
- » Salinity
- » Total Dissolved Solids
- » Resistivity
- » Temperature

Optional measurement parameters include:

- » pH
- » ORP
- » Depth

The Model 6000 is ideal for profiling and monitoring water conditions in lakes, rivers, wetlands, estuaries, coastal waters, and monitoring wells. It can be left unattended for weeks at a time with measurement parameters sampled at your setup interval and data securely saved in the unit's internal memory. The Model 6000 can be used 500 feet below the water's surface or in as little as a few inches of water. The fast sensor response of the Model 6000 and its built-in data logging make it ideal for vertical profiling. Its small size means it can fit down 4 inch diameter monitoring wells.

A new Rapid Pulse Dissolved Oxygen Sensor (patent pending) exhibits low stirring dependence and, therefore, provides accurate results without an expensive and bulky stirrer. Because no stirring is required, battery life is extended and sensor drift caused by passive fouling is minimized.

If necessary, dissolved oxygen, conductivity, temperature, pH and ORP sensors are quickly replaced in the field.



The Model 6000 communicates with an ASCII terminal or a computer with a terminal emulation program. Every Model 6000 comes with IBM compatible PC based software for simple and convenient setup and data handling. Reports and plots are automatically generated and their presentation easily customized. Data is easily exported to any spreadsheet program for more sophisticated data processing.

The RS-232C and SDI-12 interfaces provide maximum versatility for system networking and real time data collection. Several Model 6000 units are easily installed in a vertical string providing valuable water quality data at a variety of depths. For real time results, the Model 6000 can interface to radio telemetry systems and satellite, telephone, or cellular phone data collection platforms.

The Model 6000 is internally powered by eight C-size alkaline batteries that are easily replaced in the field, without disassembly of the unit. The minimal power requirements of the Rapid Pulse Dissolved Oxygen Sensor combined with state-of-the-art electronic circuits and software provide 30 days of battery life during normal use.

The Model 6000 comes with 256 kilobytes of memory; enough to store 130,000 individual readings.

The Model 6000 comes with a 25 foot cable for interfacing with a computer or terminal. The cable is waterproof at the sonde end and can be used in the lab or field. Optional underwater cables are available in a variety of lengths and are waterproof at both ends.

See Appendix D for a complete list of accessories and calibration reagents.



## **1.2 GENERAL SPECIFICATIONS**

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See also Section 1.3 Sensors Specifications.

### **Operating Environment**

Medium: fresh, sea, or polluted water

Temperature: -5 to +45 C

Depth: 0 to 500 feet

**Storage Temperature:** -40 to +60 C

**Material:** PVC, Stainless Steel

### **Dimensions**

Diameter: 3.50 inches (8.89 cm)

Length: 19.5 inches (49.53 cm)

Weight: 6.5 pounds (2.95 Kg)

### **Computer Interface**

RS-232C

SDI-12

**Software:** Works with an IBM PC compatible computer, with 3 1/2 or 5 1/4 inch, high or low density floppy disk drive.

**Minimum RAM requirement:** 256K

**Optional graphic adaptor for plotting**

**Internal logging memory size:** 256 kilobytes 130,000 individual readings

**Power:** 12 VDC - 8 C-size Alkaline Batteries (included)

**External 12 VDC (optional)**



### 1.3 SENSOR SPECIFICATIONS

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See also section 1.2 General Specifications. Performance testing of the YSI Model 6000 suggests the following typical performance.

#### Depth

Sensor Type.....Stainless steel strain gage  
Range.....0 to 500 feet  
Accuracy..... +/- 1.5 feet  
Resolution.....0.1 foot

#### Temperature

Sensor Type.....Thermistor  
Range.....-5 to 45 C  
Accuracy..... +/- 0.4 C  
Resolution.....0.1 C

#### Dissolved Oxygen % saturation

Sensor Type.....Rapid Pulse - polarographic  
Range.....0 to 200 % air saturation  
Accuracy..... +/- 2 % air saturation  
Resolution.....0.1 % air saturation

#### Dissolved Oxygen mg/L

Sensor Type.....Calculated from % air saturation, temperature and salinity.  
Range.....0 to 20 mg/L  
Accuracy..... +/- 0.2 mg/L  
Resolution.....0.01 mg/L

#### Conductivity\*

Sensor Type.....4 electrode cell  
Range.....0 to 100 mS/cm  
Accuracy..... +/- (1% of reading + 0.05 mS/cm)  
Resolution.....0.01 mS/cm or 1 uS/cm

\*Report outputs of specific conductance (conductivity corrected to 25 C), resistivity and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (ed 1989).



### **Salinity**

Sensor Type.....Calculated from conductivity and temperature

Range.....0 to 70 ppt

Accuracy..... +/-0.2 ppt

Resolution.....0.1 ppt

### **pH**

Sensor Type.....Glass combination electrode

Range.....2 to 14 units

Accuracy..... +/- 0.2 units

Resolution.....0.01 units

### **ORP**

Sensor Type.....Platinum electrode

Range.....-999 to 999 mV

Accuracy..... +/-20 mV

Resolution.....1 mV



## **1.4 HOW TO USE THIS MANUAL**

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This manual provides information for operating and servicing the YSI Model 6000 Environmental Monitoring System. Chapters 1 through 3 provide an overview of setup, calibration, and operational procedures. These first three chapters should provide enough information for you to understand the basic capabilities of the Model 6000 system and begin sampling. Chapters 4 through 9 provide a more detailed explanation of system operations, software, principles of operation, maintenance, and performance troubleshooting. Chapter 10 is written for authorized service technicians and provides necessary information for repairing the Model 6000. Appendix A-E provide information about health and safety, warranty, accessories, and options.

This manual is organized to give you a quick start into understanding and operating the YSI Model 6000 system. However, it cannot be stressed too strongly that informed and safe operation is more than just knowing which buttons to push. An understanding of the principles of operation, calibration techniques, and system setup is necessary to obtain accurate and meaningful results. Regular maintenance is required to keep the YSI Model 6000 functioning effectively. Precautions regarding the handling of reagents are also essential for the safety of system operators (see appendix A for health and safety information).

The early parts of this manual will teach you how to get the Model 6000 system running. Additional topics are included to help you understand the science it employs, how to use it most effectively and safely, and how to keep it operating correctly.



## 2. INITIAL SETUP

### 2.1 UNPACKING

Remove the instrument from the shipping container. Be careful not to discard any parts or supplies. Check off all items on the packing list and inspect all assemblies and components for damage. If any parts are damaged or missing, contact your Dealer Representative immediately. If you do not know from which dealer your YSI Model 6000 was ordered, call YSI Customer Service for assistance, see appendix C.

**Note:** Reagents for the Model 6000 are not packaged in the same carton as the instrument. These materials must be ordered separately and will arrive in a separate package.

### 2.2 SYSTEM SETUP

#### YSI 6030 DO/COND/TEMP PROBE

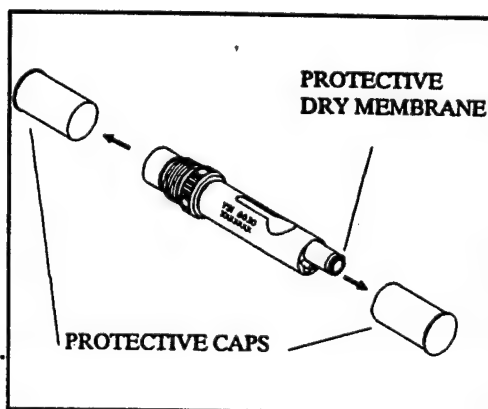
Follow these instructions to prepare your new probe and install it in the Model 6000.

1. Open the membrane kit and prepare the electrolyte. Dissolve the KCl crystals in the dropper bottle by filling it to the neck with distilled water and shaking until the solid is fully dissolved.



2. Remove the two protective caps and the dry membrane from the 6030 probe.

**NOTE:** Your probe is shipped with a protective dry membrane on the dissolved oxygen sensor tip. It is very important not to scratch or contaminate the sensor tip. Handle the new probe with care. Avoid touching or accidentally hitting the sensor tip.





3. Follow the procedure described below to install a new membrane on the dissolved oxygen sensor tip.

A. Hold the probe in a vertical position and apply a few drops of KCl solution to the tip. The fluid should completely fill the small moat around the electrodes and form a meniscus on the tip of the sensor. Be sure no air bubbles are stuck to the face of the sensor. If necessary, shake off the electrolyte and start over.

B. Secure a membrane between your left thumb and the probe body. Always handle the membrane with care, touching it at the ends only.

C. With the thumb and forefinger of your right hand, grasp the free end of the membrane. With one continuous motion, gently stretch it up, over, and down the other side of the sensor. The membrane should conform to the face of the sensor.

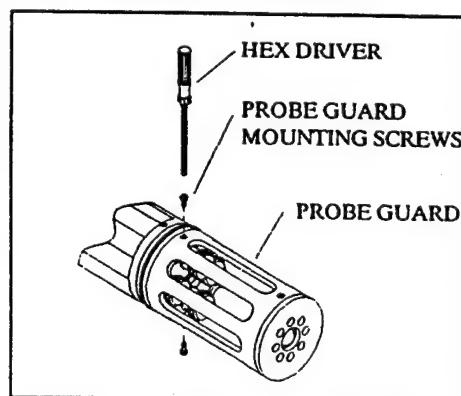
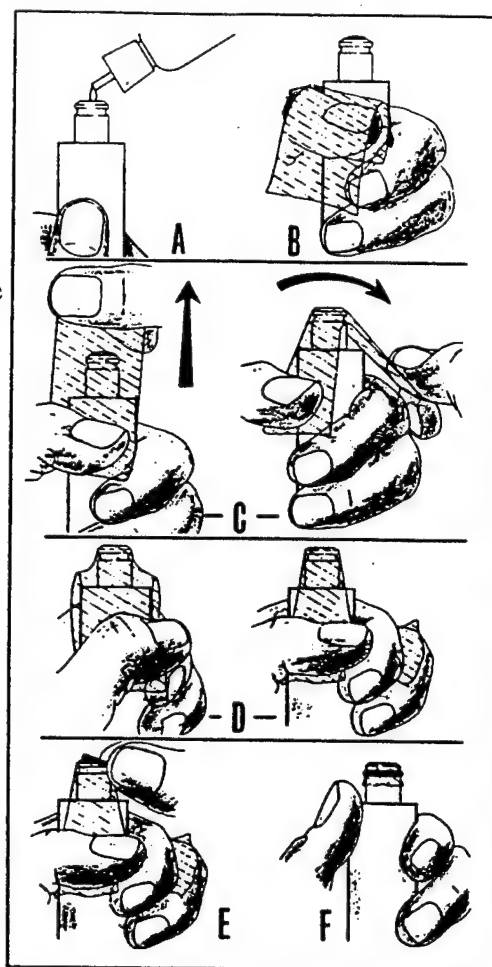
D. Secure the end of the membrane under the forefinger of your left hand.

E. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface with your fingers. There should be no wrinkles or trapped air bubbles. Small wrinkles may be removed by lightly tugging on the edges of the membrane.

F. Trim off any excess membrane with a sharp knife or scissors. Make sure the stainless steel temperature sensor is not covered by excess membrane. Being careful not to get water in the connector, rinse off the excess KCl solution.

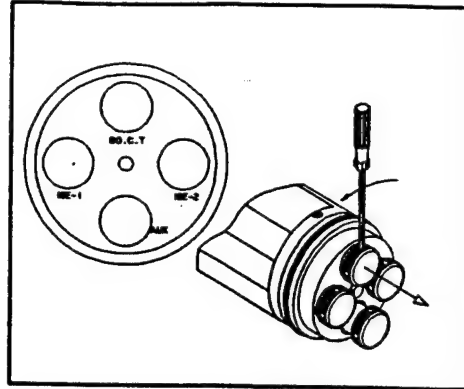
4. Using the hex driver supplied in the 6040 Maintenance Kit, remove the probe guard mounting screws from the sonde. Set the probe guard aside.

NOTE: Do not remove the two allen screws above the guard.





5. Locate the plug which seals the DO.C.T

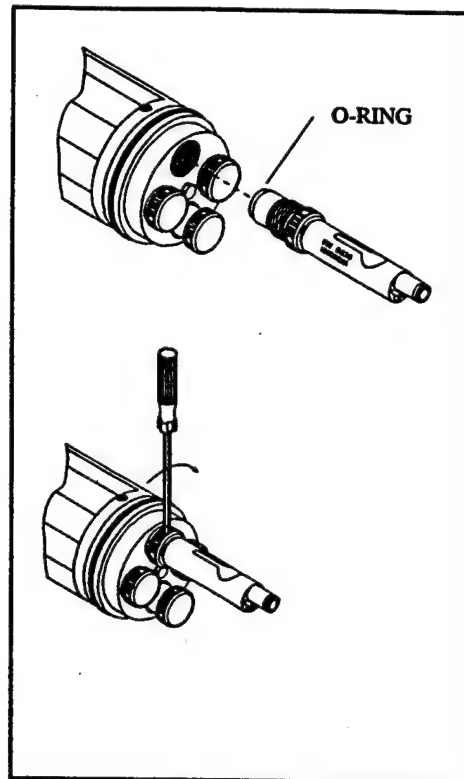


port on the sonde. Using the hex driver to assist you, remove the plug.

6. Install the 6030 probe into the sonde as described below.

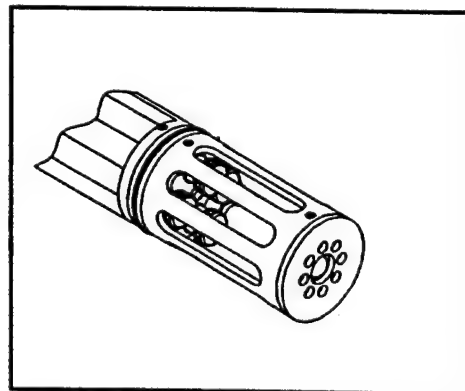
A. Apply a very thin coat of O-ring lubricant (supplied in the 6040 Maintenance Kit) to the O-ring on the connector side of the probe. Insert the probe in the port marked DO.C.T.

B. With the connectors aligned, screw down



the probe nut. Use the hex driver to assist you in tightening the nut. **DO NOT OVER TIGHTEN.**

7. Replace the probe guard and tighten the probe guard mounting screws.





## OPTIONAL PROBES

If you have purchased any optional YSI probes, follow the instruction sheet provided with your probe(s) for preparation and installation into the Model 6000. The Model 6000 comes standard with a 6030 Dissolved Oxygen / Conductivity / Temperature probe. 6031 pH, or 6032 ORP probes can be ordered as options and do not require factory installation (see section 4.7 for software setup). A depth sensor is a factory installed option.

## INSTALL BATTERIES

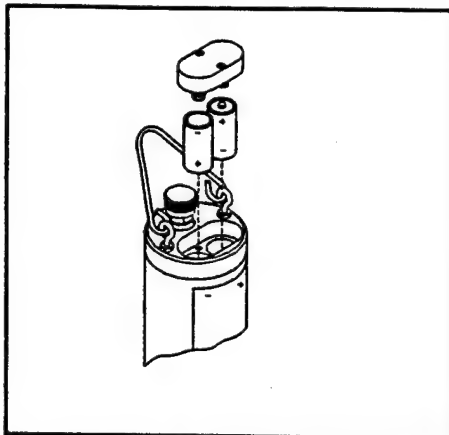
Your first set of batteries is supplied with the instrument. Install 8 C-size alkaline batteries as follows:

Use the hex driver, supplied in the 6040 Maintenance Kit, to loosen the battery lid screws.

**NOTE:** The battery lid screws are "captive", it is not necessary to remove them from the lid completely.

Remove the battery lid and install the batteries, as shown.

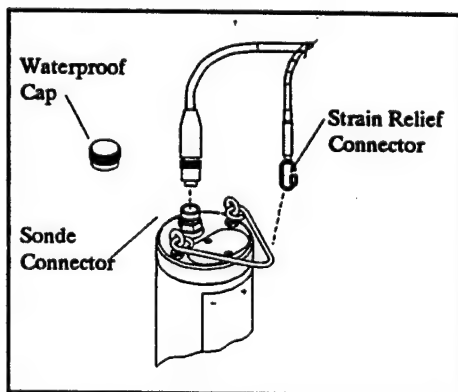
**NOTE:** Observe the correct polarity before installing the batteries into the battery chamber.



Be sure the O-ring is installed in the groove of the lid. Check the O-ring and sealing surfaces for any contaminants which could interfere with the O-ring seal of the battery chamber. Remove any contaminants present. Apply a very thin coat of O-ring lubricant (supplied in the 6040 Maintenance Kit) to the O-ring inside the battery chamber lid. Return the battery lid and tighten the screws. **DO NOT OVER TIGHTEN.**

## ATTACH CABLE

Remove the water proof cap from the sonde connector and set aside for later reassembly. Connect the PC interface cable to the sonde connector. A built-in "key" will ensure proper pin alignment. Connect the DB-25 end of the cable to an RS-232 serial port in your computer. Attach the strain relief connector to the sonde handle. Rotate the strain relief connector nut to close the connector's opening.





## 2.3 SOFTWARE INSTALLATION

---

PC6000 software is provided with every Model 6000 and is in the back of this instruction manual. Use this software if you have an IBM compatible PC with at least 256KB of RAM and DOS 3.0 or later. If your system is not IBM compatible, use any terminal emulation program with your computer.

Insert the disk into your floppy disk drive. At the C:\ prompt, type the letter of the drive in which the program disk was inserted followed by a colon, then press Enter.

Example: A: Enter.

To install PC6000 software execute the following command from the DOS prompt:

INSTALL <destination>

where "destination" is the drive and directory in which you want the PC6000 files to be installed. For example, the command:

INSTALL C:\PC6000

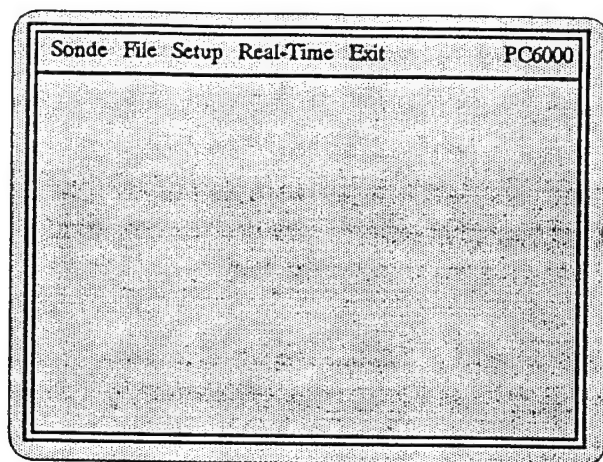
will install the PC6000 software to the C: drive and \PC6000 directory.

If you are using a two floppy disk drive system, follow the instructions in section 5.2. After installing the software, remove the disk from the floppy drive and keep the original disk in a safe place.



## 2.4 SOFTWARE SETUP

To start the software, make your current drive C:\PC6000. Type **PC6000** and press **Enter**. The PC6000 software will load. The following menu will appear at the top of the computer screen:



Use the arrow keys to move the cursor and highlight menu options. Press **Enter** to select a highlighted option. Press **Esc** to cancel an entry. To start, highlight **Setup** then press **Enter**. Check the default setup values.

Comm port:	1	Printer port:	1
Baud rate:	9600	Printer type:	Epson dot-matrix
Date format:	MDY	Menu Colors:	Scheme 1 (mono/LCD)
Date separator:	/	Plot colors:	Scheme 1 (mono)
Time separator:	:	Graphics mode:	Automatic selection hi-res
Radix mark:	.		
CDF delimiter:	,	Export file format:	PRN
		Default scaling technique:	A
Real-Time Setup		Default parameters per plot:	2
Advanced Setup			

Comm port:

Select this option to enter the number of the Comm port (1 or 2) to which the Model 6000 is connected. Be sure to press **Enter** to confirm a new entry. If the default setting is correct there is no need to change it.



#### Baud rate:

Select this option to check the baud rate. If the baud rate is not set to 9600, set it to 9600. To set the baud rate, highlight it and press **Enter**. A list of possible baud rates will appear. Select 9600 from the list and press **Enter**.

#### Printer port:

Select this option to specify the parallel port (LPT1,LPT2 or LPT3) to which your printer is connected. If no printer is connected select LPT1.

#### Printer type:

Select this option to select the type of printer connected to your computer system. From the list, choose the printer that best describes your printer. If your printer is not on the list, refer to your printer's instruction manual to determine what type of printer your printer emulates.

#### Menu colors:

Select this option to choose a color scheme for the PC6000 menus. As you move the cursor between color schemes, the screen changes to display your selection. Press **Enter** to confirm a selection.

#### Plot colors:

Select this option to choose a color scheme for the PC6000 plots. As you move the cursor between color schemes, the screen changes to display your selection. Press **Enter** to confirm a selection.

The other setup functions are described in section 5.5, but usually are not necessary for a quick start into Model 6000 operations. Press **Esc** to exit the setup menu. The cursor will return to the top line menu.

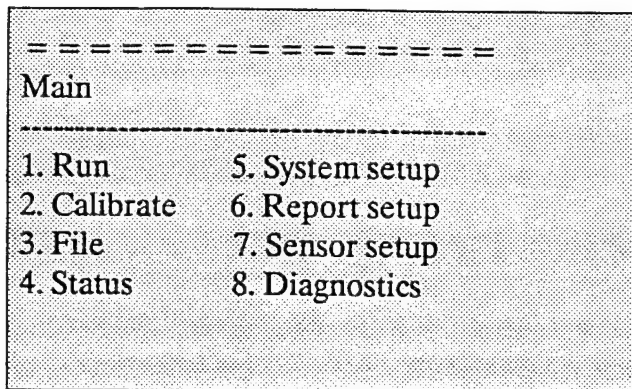


## 2.5 SONDE SETUP

---

There are two sets of software at work within the Model 6000 system. One is resident in your PC and was provided on floppy disk with this instruction manual. The other software is resident in the sonde itself. When you select **Sonde** from the PC6000 top-line menu, you are exiting the PC based software, and are entering the sonde based software.

Highlight and select **Sonde** from the top-line menu. The sonde Main menu will be displayed.

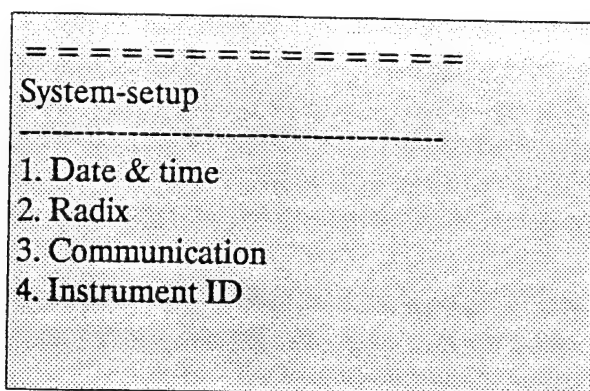


**NOTE:** If no menu is displayed and a # appears at the top of the computer screen, type **MENU** and press **Enter**. If a menu other than the Main menu is displayed, press **ESC** until the Main menu appears. If you can not get to the Main menu, check to be sure the cable is properly connected, the batteries are properly installed and the Comm port and other software parameters are setup as described in Section 2.4, or see Chapter 8, Troubleshooting.

**NOTE:** The sonde software is menu driven. Select a function by typing its corresponding number. It is not necessary to press **Enter** after a number selection. Use the **0** or **Esc** key to return to a previous menu.



At the Main menu, select **5. System setup**. The System setup menu will be displayed.



#### 1. Date & time

Select this option to choose the date format and set the correct date and time. Select the format you desire; month/day/year, day/month/year or year/month/day. Next enter the date and time as prompted on the screen.

**Note:** For convenience any non-numeric key (such as **space**) in addition to **/** and **:** will separate the fields in the date and time entries.

#### 4. Instrument ID

Select this option to identify your instrument with its serial number (located on the back of the sonde) or any other name or number you wish, up to 15 characters. Then press **Enter**.

Press **Esc** to backup to the Main menu. See Chapter 4 for a more detailed description of the sonde menus. The sonde is now setup and ready to calibrate and run. Proceed to Chapter 3.



## 3. BASIC OPERATION

---

In the previous chapter, you learned how to install probes and setup the PC6000 and sonde software. In this chapter, you will learn how to calibrate and run the Model 6000 and upload and process data.

### 3.1 CALIBRATION TIPS

---

**WARNING:** Reagents used to calibrate and check this instrument may be hazardous to your health. Refer to appendix A for health and safety information.

Before you begin the calibration procedures outlined below, you may find it helpful to follow some or all of these calibration tips.

1. Remove and retain the two allen screws at the very bottom of the sonde guard. Remove the bottom plate of the sonde guard (not the entire guard). This allows the calibration solutions access to the probes with minimal displacement of fluid within the calibration cup. Additionally, "carry-over" from one solution to the next is reduced.
2. Fill a 2 1/2 gallon bucket with cool tap water for rinsing the sonde between calibration solutions.
3. Have several clean, absorbent paper towels or cotton cloths available to dry the sonde between rinses and calibration solutions. It is important to remove as much residual liquid as possible from the sonde after each rinse. Drying the sonde and probe in this way reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration.
4. Fill each of the three calibration cups (supplied) as follows:

The first with a conductivity standard which is in the range of the water to be sampled; see the conductivity section below.

The second with a pH 7 buffer solution.

The third with a pH buffer which is in the range of the water to be sampled; see the pH section below.

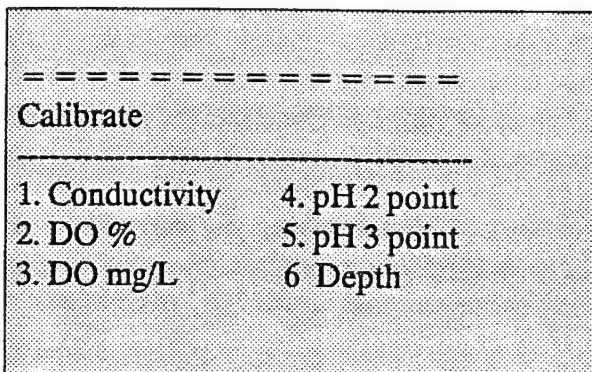


## 3.2 CALIBRATION PROCEDURES

---

**WARNING:** Calibration reagents may be hazardous to your health. Refer to appendix A for health and safety information.

From the sonde Main menu select **2. Calibrate**. The Calibrate menu will be displayed.



Three calibration cups are supplied with the Model 6000. Because the calibration cups fit over the outside of the sonde probe guard, it is not necessary to remove the guard to calibrate the sensors. Follow the procedures below to calibrate the sensors. There are two procedures for calibrating the dissolved oxygen and pH sensors. In this chapter, we only discuss **DO %** and **pH 2 point** calibration procedures. Temperature and ORP probes do not require calibration and are, therefore, not included in the Calibrate menu. For other calibration procedures, see section 4.2.



## CONDUCTIVITY -

**Note:** This procedure calibrates not only conductivity, but also specific conductance, salinity, and total dissolved solids.

Place 500 ml (approximately 1 pint) of conductivity standard in a clean calibration cup. The conductivity standard you choose should be within the same conductivity range as the water you are preparing to sample. For example:

- » For sea water choose a 50mS/cm conductivity standard.
- » For fresh water choose a 1mS/cm conductivity standard.
- » For brackish water choose a 10mS/cm conductivity standard.

Without removing the sonde guard, **slowly and carefully** immerse the probe end of the sonde into the solution. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell.

Allow at least 1 minute for temperature equilibration before proceeding.

Select **1. Conductivity** to access the Conductivity calibration procedure.

Enter SpCond in mS/cm (10.000):

Enter the calibration value of the standard you are using (mS/cm at 25 C) and press **Enter**.

**NOTE:** If an **ERROR** message appears, begin the conductivity calibration procedure again. Be certain that the value you enter for the calibration standard is correct. Also see Chapter 8, Troubleshooting.

After the calibration procedure is complete, press any key, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.



## **pH 2 POINT -**

Place 500 ml (approximately 1 pint) of pH 7 buffer in a clean calibration cup. **Slowly and carefully** immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Select **4. pH 2 point** to access the pH 2 point calibration procedure. Press any key to calibrate to pH 7.

After the pH 7 calibration is complete, press any key, as instructed on the screen, to continue.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.

Place 500 ml (approximately 1 pint) of a second pH buffer solution in a clean calibration cup. The second buffer might be pH 4 if the sample is expected to be acidic; or pH 10 if the sample is expected to be basic. **Slowly and carefully** immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Press any key to continue. When prompted on the screen, enter the value of the pH buffer (in this case, 4 or 10), and press **Enter**.

After the second value calibration is complete, press any key, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.

Thoroughly rinse and dry the calibration cups for future use.



## DISSOLVED OXYGEN -

Replace the sonde guard bottom plate, which may have been removed to simplify conductivity or pH calibration. Place a wet sponge inside a clean, empty calibration cup. Place the probe end of the sonde into the calibration cup. The oxygen probe will now be in water-saturated air.

Press **ESC** to return to the sonde Main menu. Select **1. Run** from the sonde Main menu to view the Run menu. Select **1. Discrete sample** from the Run menu to view the Run Discrete-sample menu. Select **3. Sample interval** from the Run Discrete-sample menu. Set the interval to 4 seconds and press **Enter**. Select **1. Start discrete sample** from the Run Discrete-sample menu. After 4 seconds have passed data will begin to display on the screen. Wait 10 - 15 minutes. During these 10 - 15 minutes the calibration chamber will reach 100% water-saturated air, and the D.O. sensor will warm-up and stabilize. After 10 - 15 minutes use the **ESC** key to return to the sonde Main menu.

Select **2. Calibrate** from the sonde Main menu to view the Calibrate menu. Select **2. DO %**, from the Calibrate menu, to access the DO % calibration procedure. Enter the current barometric pressure in atmospheres, e.g., 745 mm Hg is equivalent to 745/760 or 0.98 atm. Press **Enter** and the computer will indicate that the calibration procedure is in process.

After approximately 1 minute, the calibration will be complete. Press any key, as instructed on the screen, to return to the Calibrate menu.

**Note:** Calibration of dissolved oxygen in the DO % procedure also results in calibration of the DO mg/L mode and vice versa.

## DEPTH -

Keep the sonde in the air -- do not submerge it.

Select **6. Depth** to access the depth calibration procedure. Press any key to zero the depth sensor to local barometric pressure.

After depth is zeroed, press any key, as instructed on the screen, to return to the Calibrate menu.

The sensors are now calibrated. Press **Esc** until the sonde Main menu is displayed.



### 3.3 DISCRETE SAMPLING

---

Use the Discrete sampling mode to turn on or turn off the continuous sampling function. Data will be displayed to the screen and optionally to internal flash disk memory.

Select **1. Run** from the sonde Main menu. The Run menu will be displayed.

```
=====
Run
-----
1. Discrete sample
2. Conditional sample
3. Unattended sample
```

Select **1. Discrete sample** from the Run menu. The Run Discrete- sample menu will be displayed.

```
=====
Run Discrete-sample
-----
1. Start discrete sample
2. Site description
3. Sample interval
4. Close file
5. Set Auto-stop timer
```

Select the different functions in this menu to become familiar with the Discrete sampling mode. Remember to use **Esc** to cancel functions or to backup to the previous display.

```
1. Start discrete sample
```

Select this option to start discrete sampling. After the initial sampling time interval has passed, data will be displayed on the screen. A single line of data can be logged to flash disk memory by pressing **1**. (screen prompt reads '1-LOG last sample'). A set of data can be logged to flash disk by pressing **2**. (screen prompt reads '2-LOG ON/OFF'). A message will be displayed that LOG is on, turn it off by selecting **2**. Select **Esc** or **0** to exit discrete sampling.



## 2. Site description

Select this option to name the site of the sample if you intend to log data while discrete sampling. Up to 31 characters can be entered. The site description you enter will be displayed with the file name to help you identify the file later.

## 3. Sample interval

Select this option to set the interval which will pass between samples. Enter the desired interval in seconds. The default sample interval is 4 seconds.

## 4. Close file

Select this option to close any discrete sample file. A file is opened automatically whenever data is logged to flash disk memory. When you exit discrete sample mode, you can close the file using this function or you can leave it open and log to it later. If you try to setup an unattended sample study or open a new discrete sample file you will be asked if you wish to close the discrete sample file. In order to open a new file, any other open file must first be closed.

## 5. Set Auto-stop timer

Select this option to setup a timer to automatically turn off discrete sampling. If you begin a discrete sample and forget to turn it off later battery life will be greatly reduced. For this reason, the **Auto-stop timer** can be set up to automatically turn off any active discrete sample if it has been left on for a specified period of time. Simply type in what you feel is an appropriate amount of minutes, and the program will turn off discrete sampling after that many minutes have passed without activity. Setting the timer to 0 disables the Auto-stop timer, thus discrete sampling will never be stopped by the Auto-stop timer.

Press Esc until the sonde Main menu is displayed.



### 3.4 UNATTENDED SAMPLING

---

The unattended sampling mode is designed to log accurate readings of all user-defined parameters at intervals you specify. The Model 6000 can be deployed for weeks at a time using this feature. Throughout the deployment, data is stored to internal flash disk memory. This data can be uploaded to the PC6000 software upon completion of the study, or at anytime during the study.

Select **1. Run** from the sonde Main menu. The Run menu will be displayed.

```
=====
Run
-----
1. Discrete sample
2. Conditional sample
3. Unattended sample
```

Select **3. Unattended sample** from the Run menu. The current time and date, all active sensors, battery voltage, and free flash disk space will be displayed.

**NOTE:** If the current time and date are not correct, your unattended sampling study will not begin or end when you desire. To correct the time and date; see section 2.5.

You will be asked to enter the following information concerning the unattended sampling study you wish to setup.

```
Enter starting date (XX/XX/XX):
Enter starting time (XX:XX:XX):
Enter duration in days (XX):
Enter interval in minutes (1):
Enter site description ():
```



To gain experience, setup an unattended sampling study with the following responses to the above questions:

Enter starting date: *today's date*

Enter starting time: *current time*

Enter duration in days: *1*

Enter interval in minutes: *2*

Enter site description: *First-test*

You will be asked if all start-up information is correct. Check the information closely, if you want to change something press **N** and **Enter**. If all information is correct press **Y** and **Enter**. The following message will be displayed briefly:

```
*****  
*INSTRUMENT IS IN UNATTENDED MODE *  
*****
```

To complete the "first-test" you have just started, disconnect the cable from the sonde and screw on the waterproof connector cap. The unit is now ready for deployment. If the calibration cup is installed, remove it and place the Model 6000 in at least 6 inches of water. Leave it there for an hour. Proceed to the next section.

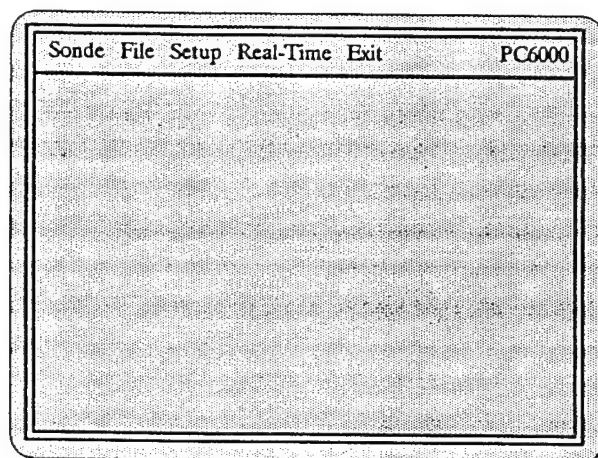


### 3.5 RECOVERING DATA FROM THE MODEL 6000

---

Attach the PC cable to the Model 6000. Connect the other end of the cable to the serial port of your computer.

Boot the PC6000 software and select **Sonde** from the top-line menu.



**NOTE:** If access to the sonde is denied, first check all cable connections and the comm port information under **Setup** of the PC6000 top-line menu. If all cable connectors are properly attached and the comm port setting is correct, it is likely that the sonde batteries have been depleted. To replace the sonde batteries see section 2.2.

From the sonde Main menu select **1. Run**.

**NOTE:** If the instrument is busy taking a sample it will display the following message: **Instrument is busy. Please wait.**

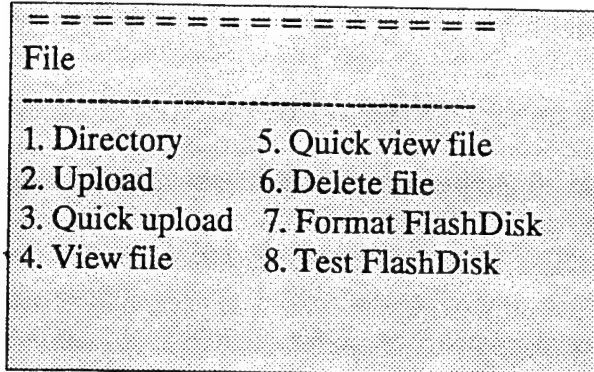
Wait until the Run menu appears and select **3. unattended sample**. The screen will display a prompt asking if you wish to cancel the unattended sampling study. Press **Y** and **Enter**.

**NOTE:** Data can be uploaded while an unattended sampling study is in progress.

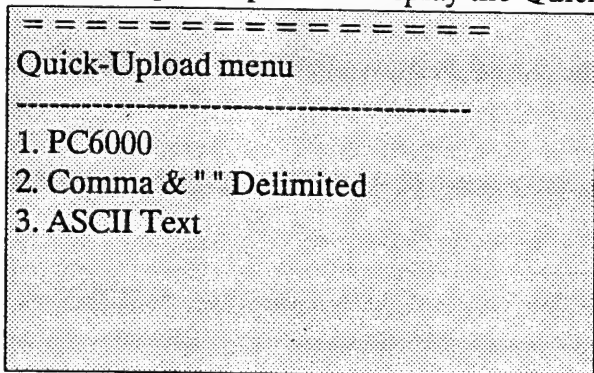
**NOTE:** If unattended sampling has already stopped automatically because the "duration" you entered earlier has expired, press **ESC** twice to return to the Main menu.



Return to the sonde Main menu using the **Esc** key. Select **3. File** to display the File menu.



Select **3. Quick upload** to display the Quick-Upload menu.



Select **1. PC6000** to upload to the PC6000 software. A secondary menu will appear to show the progress of the upload.

**NOTE:** Comma & " " Delimited and ASCII Text formats are available for uploading data to a spreadsheet or database program. See section 4.3 for more details.

When the upload is complete, press **Esc** until the sonde Main menu returns. Press **F10** to exit the sonde menu and return to the PC6000 top-line menu.



### 3.6 LOADING A FILE

Select **File** from the top-line menu.

Sonde **File** Setup Real-Time Exit YSI PC6000

A box will appear on the screen asking you to enter a filename.

Filename: \*.dat

You may type a file name or press **Enter** for a directory. If the directory contains more than one data file, a list of files will be displayed. Use the arrow keys to highlight and the **Enter** key to select the file of choice.

**NOTE:** If only one data file is resident in the directory, it will be automatically loaded.

After the selected file has been retrieved, the following menu will be displayed:

MODIFY	VIEW	SYSTEM
Parameters	Header	Print Report
Control Info	Report	Print Plot
Scaling Info	Plot	Definitions
	Statistics	Export

Any changes made from this menu will effect only the current file. To change default values for all files, see section 5.5.

Header

Select this option to view general information about the file. Information displayed on the header screen includes the file name, site description, a list of active sensors, and other details about this file. Press **Esc** to return to the File menu.



## Report

Select this option to view a time-stamped report of the data collected during the study. Press **Esc** to return to the File menu.

## Plot

Select this option to view a plot of the data collected during the study. Press **Esc** until the top-line menu of the PC6000 program appears.

All plots and reports can be customized to your specific requirements, see section 5.4 for details.



### **6.3 OXIDATION REDUCTION POTENTIAL (ORP)**

---

The Model 6000 determines the Oxidation-Reduction Potential (ORP) of the media by measuring the difference in potential between an electrode which is relatively chemically inert and a reference electrode. The Model 6000 utilizes a field-replaceable combination probe with a platinum tip and an Ag/AgCl reference electrode which utilizes gelled electrolyte. ORP values are presented in millivolts and are not compensated for temperature.

#### **CALIBRATION**

No calibration as such is required for the ORP readings of the Model 6000. However, to determine if the probe is functioning properly, the sensor can be immersed in a solution of known ORP. A solution consisting of a mixture of potassium ferricyanide and potassium ferrocyanide in 0.1 M KCl (ZoBell solution) is available from YSI for this accuracy check. The ORP output should be in the 221 to 241 mV range for the ZoBell solution if the ORP probe is within the manufacturer's specifications.

#### **MEASUREMENT PRECAUTIONS**

- (1) Clean and store the probe according to the manufacturer's instructions.



## **Appendix B-5 – Lab Procedures for Total Kjeldahl Nitrogen (TKN)**



## Table of Contents – Appendix B-5

Documents
QC Document – Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAI)
LACHAT Instruments – Quik Chem Method 10-107-06-2-D
LACHAT Instruments – Quik Chem Method 10-107-06-2-E
Technicon Auto Analyzer II – Industrial Method No. 334-74W/B



## Nitrogen, Total Kjehldahl - Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAI)

### 1.0 Procedure

Perform analysis for Total Kjehldahl Nitrogen (Method 351.2) in accordance with procedures for the Technicon II AutoAnalyzer, or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



# NITROGEN, KJELDAHL, TOTAL

## Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAI)

STORET NO. 00625

1. Scope and Application
  - 1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogeneous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/1 TKN. The range may be extended with sample dilution.
2. Summary of Method
  - 2.1 The sample is heated in the presence of sulfuric acid,  $K_2SO_4$  and  $HgSO_4$  for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.
3. Definitions
  - 3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate  $(NH_4)_2SO_4$ , under the conditions of digestion described below.
  - 3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
  - 4.1 Samples may be preserved by addition of 2 ml of conc  $H_2SO_4$  per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.
5. Apparatus
  - 5.1 Block Digester-40
  - 5.2 Technicon Manifold for Ammonia (Figure 1)
  - 5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)
6. Reagents
  - 6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide ( $HgO$ ) in 50 ml of 1:4 sulfuric acid (10 ml conc  $H_2SO_4$ ; 40 ml distilled water) and dilute to 100 ml with distilled water.
  - 6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of  $K_2SO_4$  in 700 ml of distilled water and 200 ml of conc  $H_2SO_4$ . Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Pending approval for NPDES  
Issued 1978



- 6.3 Sulfuric Acid Solution (4%): Add 40 ml of conc. sulfuric acid to 800 ml of ammonia free distilled water, cool and dilute to 1 liter.
- 6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 ml of ammonia-free distilled water and dilute to 1 liter.
- 6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.
- 6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic ( $\text{Na}_2\text{HPO}_4$ ) in about 800 ml of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.
- 6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (6.5) to 200 ml of stock buffer solution (6.6) and mix. Add xx ml sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.
- 6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.
- 6.9 Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled water.
- 6.10 Ammonium chloride, stock solution: Dissolve 3.819 g  $\text{NH}_4\text{Cl}$  in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml = 1.0 mg  $\text{NH}_3\text{-N}$ .

## 7. Procedure

### Digestion

- 7.1 To 20 or 25 ml of sample, add 5 ml of digestion solution (6.2) and mix (use a vortex mixer).
- 7.2 Add (4–8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.
- 7.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digester and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.
- 7.4 Cool sample and dilute to 25 ml with ammonia free water.

### Colorimetric Analysis

- 7.5 Check the level of all reagent containers to ensure an adequate supply.
- 7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.
- 7.7 Flush the Sampler IV wash receptacle with about 25 ml of 4.0% sulfuric acid (6.3).
- 7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.



TABLE 1  
CONCENTRATION RANGES  
(NITROGEN)

No.	Initial sample		Dilution loops		Resample	Approx. std. cal. setting	Range PPM N ( $\pm 10\%$ )	ml stock NaOH per liter working buffer solution
	Sample line	Diluent line	Diluent line	Resample line				
1	.80 (RED/RED)	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0-2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0-10	80

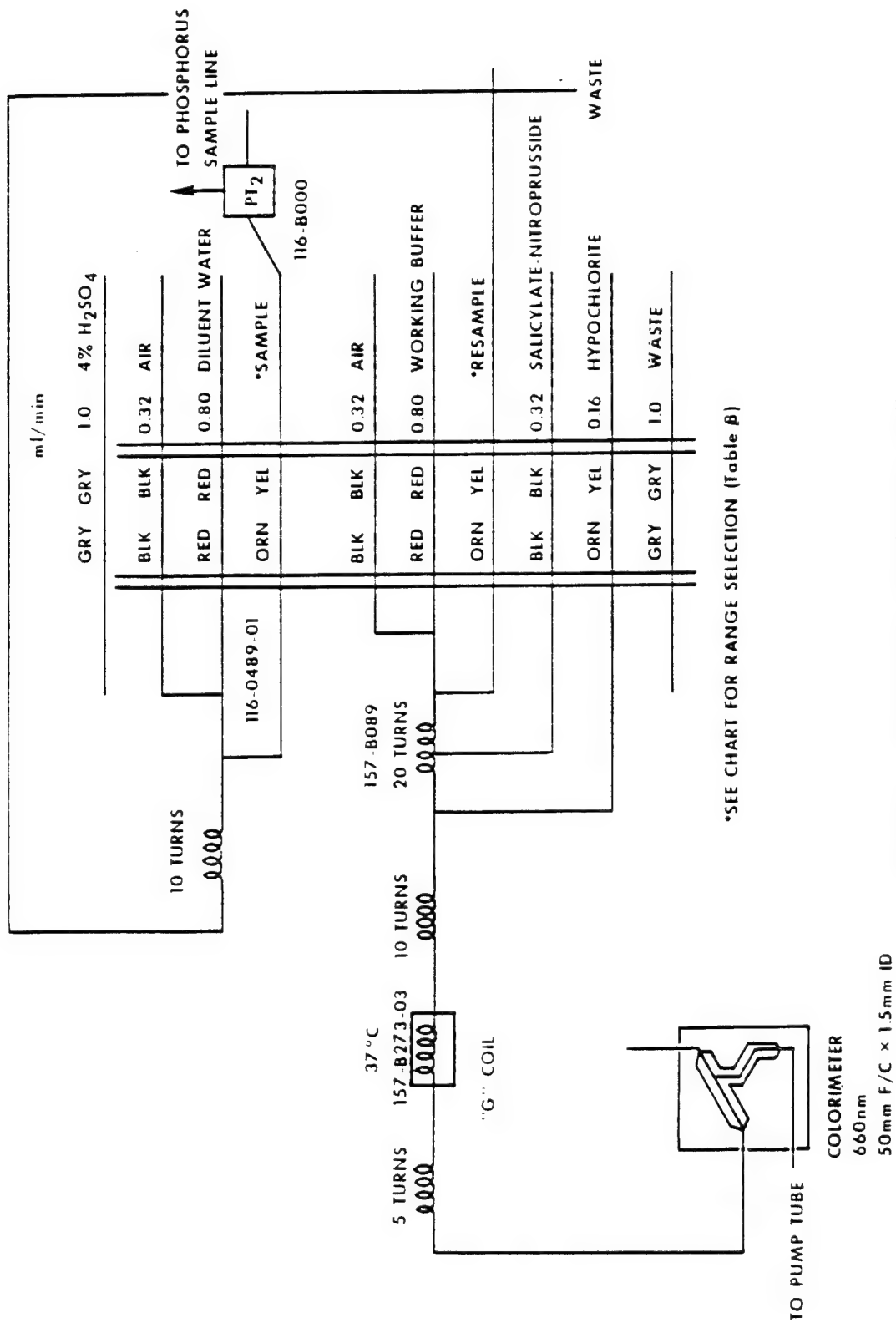


- 7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.
- 7.10 After a stable baseline has been obtained start the Sampler.
- 8. Calculations
  - 8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.
- 9. Precision and Accuracy
  - 9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/l, the precision was  $\pm 0.07$ ,  $\pm 0.03$  and  $\pm 0.15$ , respectively.
  - 9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/l, the recoveries were 99 and 99%, respectively.

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- 2. Gales, M.E., and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.
- 3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May, 1974.
- 4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August, 1974.
- 5. Gales, M.E., and Booth, R.L., "Evaluation of the Block Digestion System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600/4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.





\*SEE CHART FOR RANGE SELECTION (Table β)

FIGURE 1. AMMONIA MANIFOLD AAI



**QuikChem METHOD 10-107-06-2-D**

**DETERMINATION OF TOTAL KJELDAHL NITROGEN BY**  
**FLOW INJECTION ANALYSIS COLORIMETRY**  
**(BLOCK DIGESTOR METHOD)**

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS  
6645 WEST MILL ROAD  
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# LACHAT

INSTRUMENTS

QuikChem Method 10-107-06-2-D

## Total Kjeldahl Nitrogen in Waters

0.2 to 20 .0 mg N/L

### -- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

### -- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H<sub>2</sub>SO<sub>4</sub> in the diluted digestion sample with no change in signal intensity.
2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

### -- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers



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## DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

### 1. SCOPE AND APPLICATION

- 1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.
- 1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.
- 1.3. The applicable range is 0.2 to 20 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.
- 1.4. Samples containing particulates should be filtered or homogenized.

### 2. SUMMARY OF METHOD

- 2.1. The sample is heated in the presence of sulfuric acid,  $H_2SO_4$ , for two and one half hours. The residue is cooled, diluted with water and analyzed for ammonia. This digested sample may also be used for phosphorus determination.
- 2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate  $(NH_4)_2SO_4$ , under the conditions of the digestion described.
- 2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.
- 2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.
- 2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.



### **3. DEFINITIONS**

- 3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.
- 3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.
- 3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.
- 3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of



calibration standards. It is used to check laboratory performance with externally prepared test materials.

- 3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

#### 4. INTERFERENCES

- 4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H<sub>2</sub>SO<sub>4</sub> in the diluted digestion sample with no change in signal intensity.
- 4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.
- 4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

#### 5. SAFETY

- 5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.
- 5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.
- 5.3.1. Mercury (Reagents 1 and 2)
- 5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)
- 5.3.3. Sodium Nitroprusside (Reagent 4)



## **6. EQUIPMENT AND SUPPLIES**

- 6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.
- 6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
  - 6.3.1. Sampler
  - 6.3.2. Multichannel proportioning pump
  - 6.3.3. Reaction unit or manifold
  - 6.3.4. Colorimetric detector
  - 6.3.5. Data system
- 6.4. Special apparatus
  - 6.4.1. Heating Unit
  - 6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)
  - 6.4.3. 5 mL and 20 mL repipet dispensers
  - 6.4.4. Vortex mixer

## **7. REAGENTS AND STANDARDS**

### **7.1. PREPARATION OF REAGENTS**

Use deionized water (10 megohm) for all solutions.

#### **Degassing with Helium**

To prevent bubble formation, the water carrier is degassed with helium. Use He at 20 lb/in<sup>2</sup> through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degassed.



#### Reagent 1.      Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 40.0 mL water and 10 mL concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ). Then add 8.0 g red mercuric oxide ( $\text{HgO}$ ). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

#### Reagent 2.      Digestion Solution

In a 1 L volumetric flask, add 133.0 g potassium sulfate ( $\text{K}_2\text{SO}_4$ ) and 200 mL concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to approximately 700 mL water. Add 25.0 mL Reagent 1. Dilute to the mark with water and invert to mix. Prepare fresh monthly.

#### Reagent 3.      Buffer

**By Volume:** In a 1 L volumetric flask containing 900 mL water completely dissolve 30.0 g sodium phosphate dibasic heptahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ). Next, add 17.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 65 g sodium hydroxide ( $\text{NaOH}$ ), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

**By Weight:** To a tared 1 L container add 958 g water and completely dissolve 30.0 g sodium phosphate dibasic heptahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ). Next, add 17.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 65 g sodium hydroxide ( $\text{NaOH}$ ). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

#### Reagent 4.      Salicylate Nitroprusside

**By Volume:** In a 1 L volumetric flask dissolve 150.0 g sodium salicylate [salicylic acid sodium salt,  $\text{C}_6\text{H}_4(\text{OH})(\text{COO})\text{Na}$ ], and 1.00 g sodium nitroprusside [sodium nitroferricyanide dihydrate,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ ] in about 800 mL water. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

**By Weight:** To a tared 1 L dark container, add 150.0 g sodium salicylate [salicylic acid sodium salt,  $\text{C}_6\text{H}_4(\text{OH})(\text{COO})\text{Na}$ ], 1.00 g sodium nitroprusside [sodium nitroferricyanide dihydrate,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ ] and 908 g water. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.



**Reagent 5. Hypochlorite Solution**

**By Volume:** In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

**By Weight:** To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

**Reagent 6. Diluent 5.0% (V/V) Sulfuric Acid**

**NOTE:** Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

**By Volume:** In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

**By Weight:** To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2** (Digestion Solution). Invert to mix.



## 7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

### Digested Standards

**NOTE:** Working standards prepared in DI water are digested per the procedure in section 8.

#### Standard 1: Stock Standard 1000 mg N/L

In a 1 L volumetric flask dissolve 3.819 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) that has been dried for two hours at  $110^\circ\text{C}$  in about 800 mL DI water. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500.

#### Standard 2: Working Stock Standard 20.0 mg N/L

**By Volume:** In a 250 mL volumetric flask, dilute 5.0 mL Stock Standard 1 to the mark with DI water. Invert to mix.

**By Weight:** To a tared 1 L container add about 20 g Stock Standard 1. Divide the exact weight of the standard solution by 0.02 and dilute up to this resulting total weight with DI water. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

#### By Volume

Volume (mL) of Standard 2 diluted to 100 mL with DI water	100	50	25	10	5	2.5	0
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#### By Weight

Weight (g) of Standard 2 diluted to final weight (~250 g) divide by factor below with DI water.	250.0	125	62.5	25	12.5	6.25	0
Division Factor	1.00	0.50	0.25	0.10	0.05	0.025	0
Divide exact weight of the standard by this factor to give final weight							



## Non-Digested Standards

### Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

**By Volume:** In a 1 L volumetric flask containing approximately 600 mL water, add 250 mL Reagent 2 (Digestion Solution). Dilute to the mark and invert to mix.

**By Weight:** To a tared 1 L container, add 760 g water and 250 mL Reagent 2 (Digestion Solution). Invert to mix.

### Standard 4. High Standard in Digestion Matrix (20.0 mg N/L)

**By Volume:** In a 1 L volumetric flask containing approximately 600 mL water, add 250 mL Reagent 2 (Digestion Solution). Add 20 mL of Standard 1 (1000 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

**By Weight:** To a tared 1 L container, add 740 g water and 250 mL Reagent 2 (Digestion Solution). Add 20 g of Standard 1 (1000 mg N/L) and shake to mix.

**Note:** Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.500	0.00

#### By Volume

Volume (mL) of Standard 2 diluted to 100 mL with Reagent 6	100	50	25	10	5	2.5	0
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#### By Weight

Weight (g) of Standard 2 diluted to final weight (~ 250 g) divide by factor below with Reagent 6.	250.0	125	62	25	12.5	6.25	0
Division Factor	1.00	0.50	0.25	0.10	0.05	0.025	0
Divide exact weight of the standard by this factor to give final weight							



## **8. SAMPLE COLLECTION, PRESERVATION AND STORAGE**

- 8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.
- 8.2 Samples should be preserved to  $\text{pH} < 2$  and cooled to  $4^{\circ}\text{C}$  at the time of collection.
- 8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at  $4^{\circ}\text{C}$  and may be held for up to 28 days.

## **9. QUALITY CONTROL**

- 9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

### **9.2 INITIAL DEMONSTRATION OF PERFORMANCE**

- 9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.
- 9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being  $> 10\text{X MDL}$ . If any determined concentration exceeds the known values by  $\pm 10\%$ , linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.
- 9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within  $\pm 10\%$  of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.



- 9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where,  $t$  = Student's  $t$  value for a 99% confidence level and a standard deviation estimate with  $n-1$  degrees of freedom [ $t = 3.14$  for seven replicates,  $t = 2.528$  for twenty one replicates].

$S$  = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

### 9.3. ASSESSING LABORATORY PERFORMANCE

- 9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.
- 9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.3.3. The laboratory must use LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery ( $\bar{X}$ ) and the standard deviation ( $S$ ) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \bar{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation ( $S$ ) data should be used to establish an on-going



precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

- 9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

#### 9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

- 9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

- 9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

Where,

R = percent recovery

C<sub>s</sub> = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

- 9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.



- 9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

## **10. CALIBRATION AND STANDARDIZATION**

- 10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).
- 10.4. Calibrate the instrument as description in section 11.
- 10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.
- 10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed  $\pm 10\%$  of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

## **1. PROCEDURE**

### **11.1. DIGESTION PROCEDURE**

**NOTE:** Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

- 11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.
- 11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)



- 11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.
- 11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digester for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.
- 11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.
- 11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.
- 11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.
- 11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

## 11.2. SYSTEM START-UP PROCEDURE

- 11.2.1. Prepare reagent and standards as described in section 7.
- 11.2.2. Set up manifold as shown in section 17.1.
- 11.2.3. Input peak timing and integration window parameters as specified in section 17.
- 11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.
- 11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.
- 11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).



## 11.4. SYSTEM NOTES

- 11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.
- 11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.
- 11.4.3. If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.
- 11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.
- 11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.
- 11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.
- 11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:
  - A. Place transmission lines in water and pump to clear reagents (2-5 minutes).
  - B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.
  - C. Place all transmission lines in water and pump for several minutes.
  - D. Resume pumping reagents.

## 12. DATA ANALYSIS AND CALCULATIONS



- 12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.
- 12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3. Report results in mg N/L.

### **13. METHOD PERFORMANCE**

- 13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

### **14. POLLUTION PREVENTION**

- 14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.
- 14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy." 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

### **15. WASTE MANAGEMENT**

- 15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and



by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

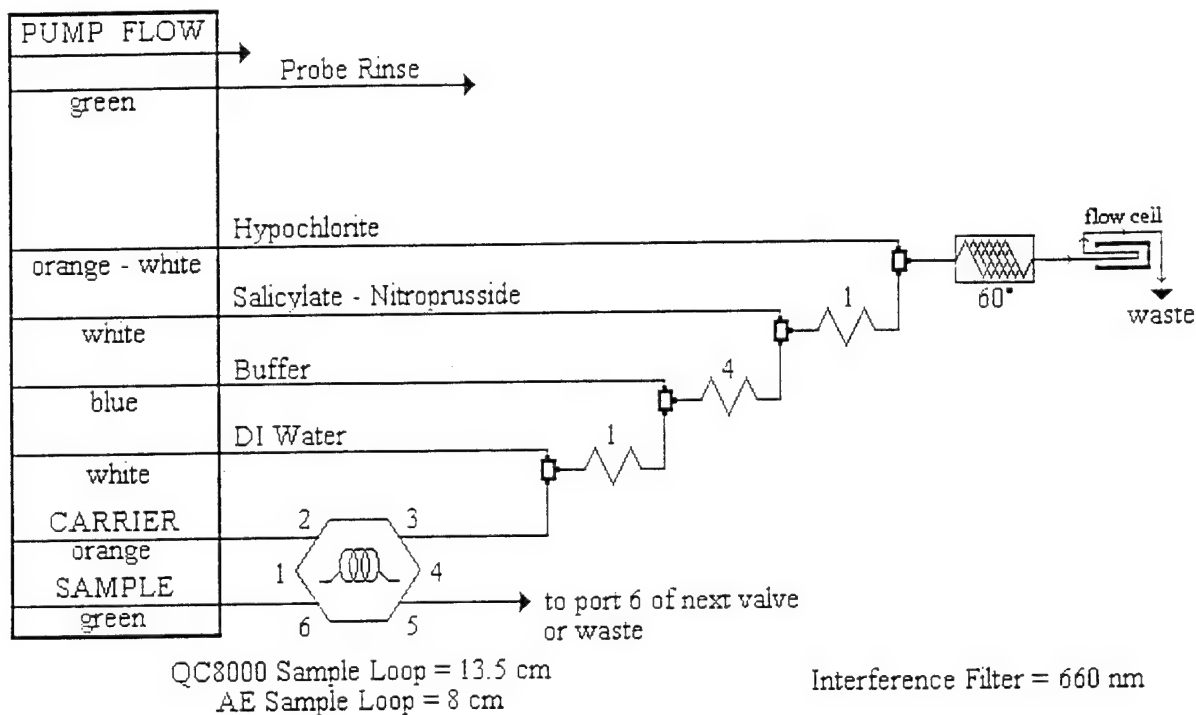
## 16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.



## 17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

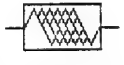
### 17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is 70 cm of tubing on a 1 inch coil support

4 is 255 cm of tubing on a 4 inch coil support

**Apparatus:** Standard valve, flow cell, and detector head modules are used. The  shows 650 cm of heated tubing. All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc



## 17.2 DATA SYSTEM PARAMETERS FOR THE QUIKCHEM AE

Sample throughput: 90 samples/hour; 60 s/sample  
Pump speed: 35  
Cycle Period: 45 s

Inject to start of peak period: 38 s

Presentation, Data Window

Top Scale Response: 0.32 abs

Bottom Scale Response: 0.00 abs

Segment/Boundaries: A: 20.00 mg N/L

E: 1 mg N/L

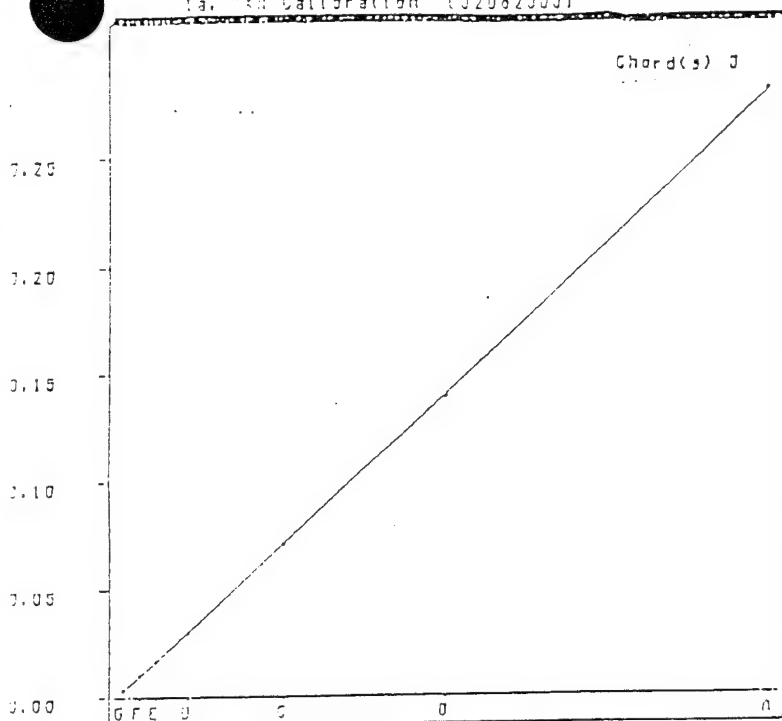
F: 0.00 mg N/L

Series 4000/System IV Settings: Gain = 420 x 1



# 17.3 QUIKCHEM AE SUPPORT DATA

1a. IKN Calibration (02062003)



Std ug H/L  
 A 20.00  
 B 10.00  
 C 5.00  
 D 2.00  
 E 1.00  
 F 0.50  
 G 0.00

Calibration Statistics Report  
 Cal Ref: 92062003 06/24/92 03:18 pm  
 Method: IKN-F

Channels: IKN

Correlation Coefficients

Std	Std	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.9997	0.9998	0.9998	0.9999	0.9999	0.9999
2	E-G	0.9997	0.9998	0.9998	0.9999	0.9999	0.9999

Percent Standard Deviation in Slope

1	A-E	0.7	1.5	0.2	0.1	0.1	0.1
2	E-G	1.6	31.5	0.8	0.8	0.8	0.8

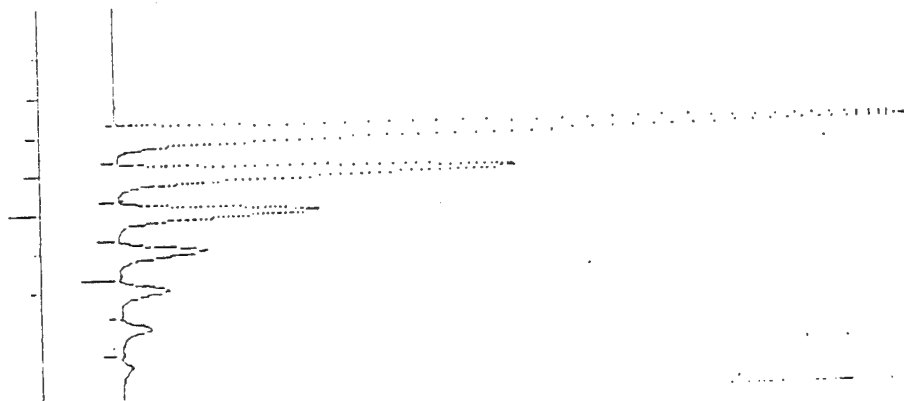
QUIKCHEM AE Calibration Report for Calibration 02062003  
 Method: IKN

This calibration was done on 06/23/92 at 07:51 pm  
 This report prepared on 06/24/92 at 03:44 pm

Standard	Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
			Known	Determined	Residual	
Standard A, IKN	ug H/L		20.000	24.512	-0.21	0.2755
Standard B, IKN	ug H/L		10.000	1.513	0.87	0.1374
Standard C, IKN	ug H/L		5.000	4.375	0.12	0.0719
Standard D, IKN	ug H/L		2.000	2.597	-0.35	0.0381
Standard E, IKN	ug H/L		1.000	1.399	0.09	0.0168
Standard F, IKN	ug H/L		0.500	1.590	-0.81	0.0101
Standard G, IKN	ug H/L		0.000	-0.890	0.00	0.0033

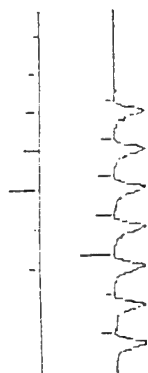


1. Standard A (1)
2. Standard B (1)
3. Standard C (1)
4. Standard D (1)
5. Standard E (1)
6. Standard F (1)
7. Standard G (1)



Back 1 (Ref: 92062300) 06/23/1992, 08:08 pm

11. 0.5 ug N/L (1)
12. 0.5 ug N/L (1)
13. 0.5 ug N/L (1)
14. 0.5 ug N/L (1)
15. 0.5 ug N/L (1)
16. 0.5 ug N/L (1)
17. 0.5 ug N/L (1)



Cup# Sample ID

101	0.5 mg N/L
102	0.5 mg N/L
103	0.5 mg N/L
104	0.5 mg N/L
105	0.5 mg N/L
106	0.5 mg N/L
107	0.5 mg N/L

1  
TKN  
mg N/L

0.492<  
0.496<  
0.506  
0.492<  
0.479<  
0.487<  
0.503

MDL

mean = 0.494  
s = 0.0092  
(3.14) s = 0.029 mg N/L

Back 1 (Ref: 92062306) 06/23/1992, 07:51 pm

101. Blank 4.8% H2SO4 (1)
102. Blank 4.8% H2SO4 (1)
103. Blank 4.8% H2SO4 (1)
104. Blank 4.8% H2SO4 (1)
105. Blank 4.8% H2SO4 (1)
106. Blank 4.8% H2SO4 (1)
107. Blank 4.8% H2SO4 (1)
108. Blank 4.8% H2SO4 (1)
109. Blank 4.8% H2SO4 (1)
110. Blank 4.8% H2SO4 (1)



Cup# Sample ID

101	Blank 4.8% H2SO4
102	Blank 4.8% H2SO4
103	Blank 4.8% H2SO4
104	Blank 4.8% H2SO4
105	Blank 4.8% H2SO4
106	Blank 4.8% H2SO4
107	Blank 4.8% H2SO4
108	Blank 4.8% H2SO4
109	Blank 4.8% H2SO4
110	Blank 4.8% H2SO4

1  
TKN  
mg N/L

0.010<  
-0.004<  
0.007<  
0.012<  
-0.003<  
-0.000<  
0.021<  
0.008<  
0.015<  
-0.007<

EMDL DATA

Mean = 0.0059  
s = 0.0091  
(4.65) s = 0.04 mg N/L

Back 1 (Ref: 92062310) 06/23/1992, 09:29 pm

101. 20 ug N/L (1)
102. Blank 4.8% H2SO4 (1)
103. Blank 4.8% H2SO4 (1)
104. Blank 4.8% H2SO4 (1)
105. Blank 4.8% H2SO4 (1)
106. Blank 4.8% H2SO4 (1)
107. Blank 4.8% H2SO4 (1)
108. Blank 4.8% H2SO4 (1)



Cup# Sample ID

101	20 mg N/L
102	Blank 4.8% H2SO4
103	Blank 4.8% H2SO4
104	Blank 4.8% H2SO4
105	Blank 4.8% H2SO4
106	Blank 4.8% H2SO4
107	Blank 4.8% H2SO4
108	Blank 4.8% H2SO4

1  
TKN  
mg N/L

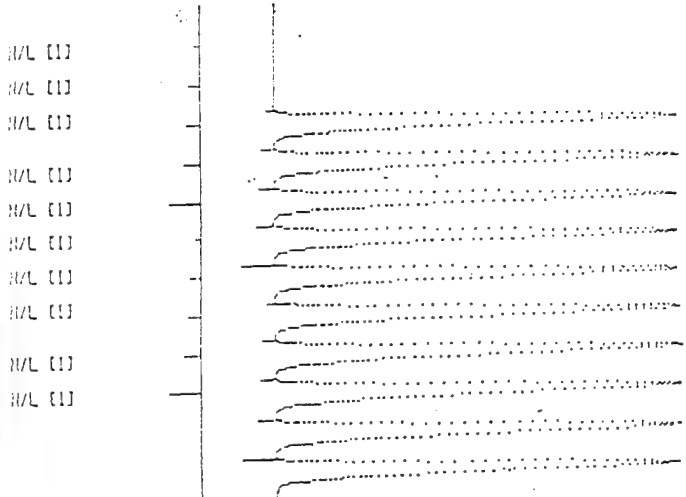
20.239  
-0.014<  
-0.012<  
0.003<  
-0.022<  
0.001<  
0.012<  
0.012<

Carry-over

mean = -0.0029  
s = 0.0133  
95% CI = -0.015 to 0.009  
(passes)



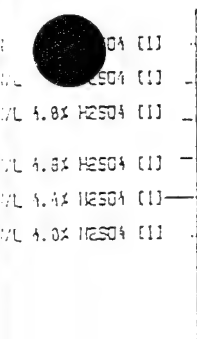
(Ref: 92062309) 06/23/1992, 08:15 pm



Cup#	Sample ID	TKN mg N/L
101	10.0 mg N/L	10.017
102	10.0 mg N/L	10.022
103	10.0 mg N/L	9.971
104	10.0 mg N/L	9.989
105	10.0 mg N/L	10.001
106	10.0 mg N/L	10.096
107	10.0 mg N/L	10.125
108	10.0 mg N/L	10.102
109	10.0 mg N/L	10.068
110	10.0 mg N/L	10.065

Precision  
-----  
mean = 10.06  
s = 0.065  
VRSD = 0.64

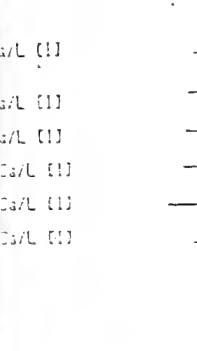
(Ref: 92062307) 06/23/1992, 08:00 pm



Cup#	Sample ID	TKN mg N/L
101	5.0 mg N/L 4.0% H2SO4	5.039
102	5.0 mg N/L 4.4% H2SO4	4.093
103	5.0 mg N/L 4.0% H2SO4	4.753
104	5.0 mg N/L 4.0% H2SO4	5.011
105	5.0 mg N/L 4.4% H2SO4	4.025
106	5.0 mg N/L 4.0% H2SO4	4.757

Acid Effect  
-----  
mean (4.8%) = 5.025 mg N/L  
mean (4.4%) = 4.859 mg N/L  
mean (4.0%) = 4.775 mg N/L

(Ref: 92062311) 06/23/1992, 08:33 pm



Cup#	Sample ID	TKN mg N/L
101	20 mg Ca/L	-0.002<
102	20 mg Ca/L	0.001<
103	20 mg Ca/L	0.021<
104	100 mg Ca/L	0.044<
105	100 mg Ca/L	0.051<
106	100 mg Ca/L	0.080<

Calcium Interference  
-----  
<0.10 mg N/L at 100 mg Ca/L



## 17.4 DATA PARAMETERS FOR THE QUIK CHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput: 90 samples/hour; 60 s/sample  
Pump speed: 35  
Cycle Period: 45 s

### Analyte data:

Peak Base Width: 39 s  
% Width Tolerance: 100  
Threshold: 11537  
Inject to Peak Start: 42 s  
Chemistry: Direct

### Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

### Sampler Timing:

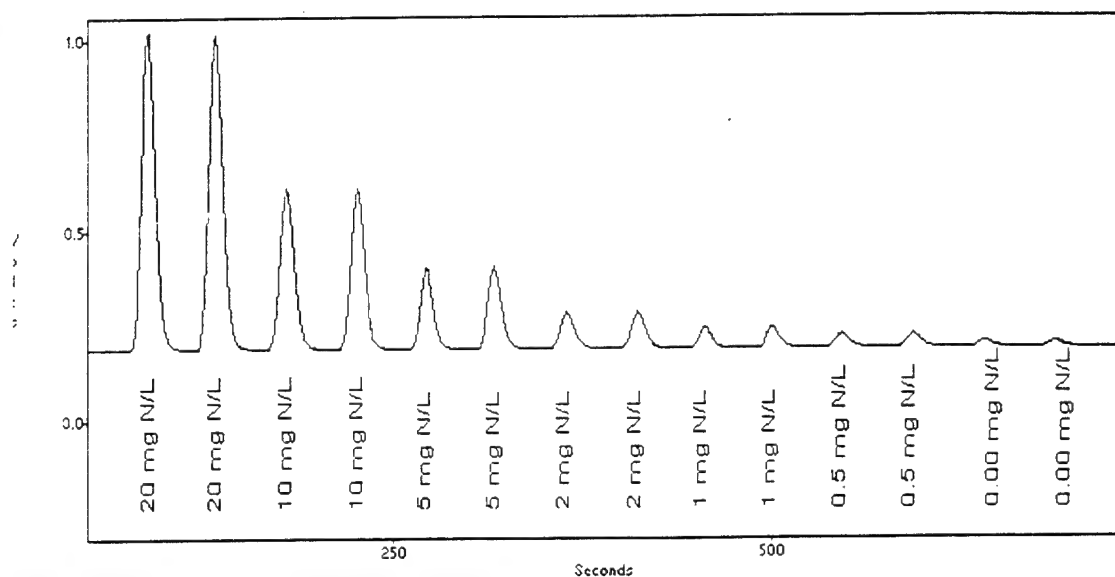
Min. Probe in Wash Period: 14 s  
Probe in Sample Period: 20 s

### Valve Timing:

Load Period: 20 s  
Inject Period: 25 s  
Load Time: 0.0 s



# 17.5 QUIKCHEM 8000 SUPPORT DATA



ACQ. TIME: Aug 22, 1994 13:25:57  
 DATA FILENAME: CAOMNION\DATA\1010762E\082294C2.FDT  
 METHOD FILENAME: CAOMNION\METHODS\1010762D\1010762D.MET

## Calibration Graph and Statistics

Level	Area	mg CN <sup>-</sup> /L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8596849	20	20.000	8596849	8634613	26703.2	0.3	-0.0
2	4383597	10	10.020	438597	4373046	7460.7	0.2	-0.2
3	2248447	5	4.960	2248447	2246723	1218.7	0.1	0.8
4	990856	2	1.991	990856	978804	8522.1	0.9	0.9
5	574638	1	0.997	574638	566821	5527.9	1.0	0.3
6	366814	0.5	0.504	366814	364718	1481.9	0.4	-0.9
7	167977	0	0	167977	165993	1403.2	0.8	---

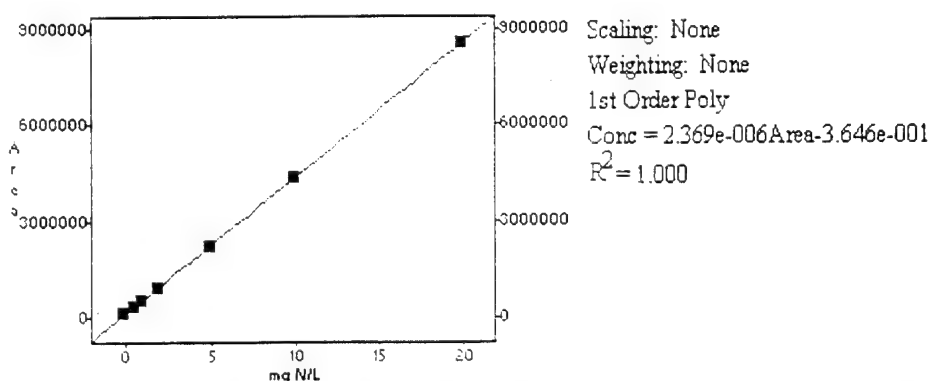
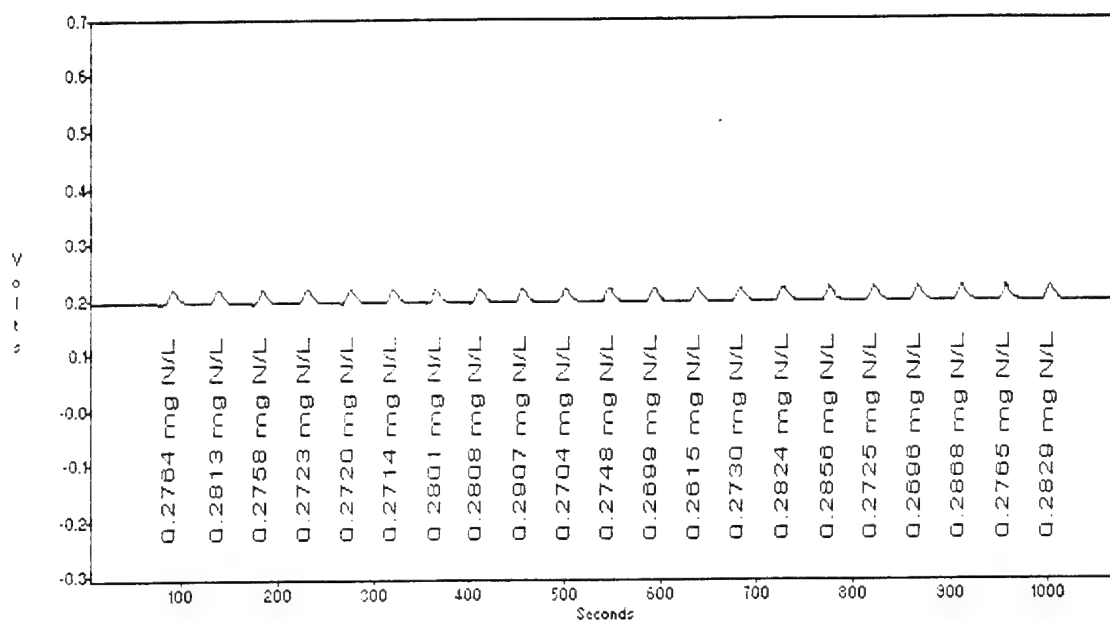




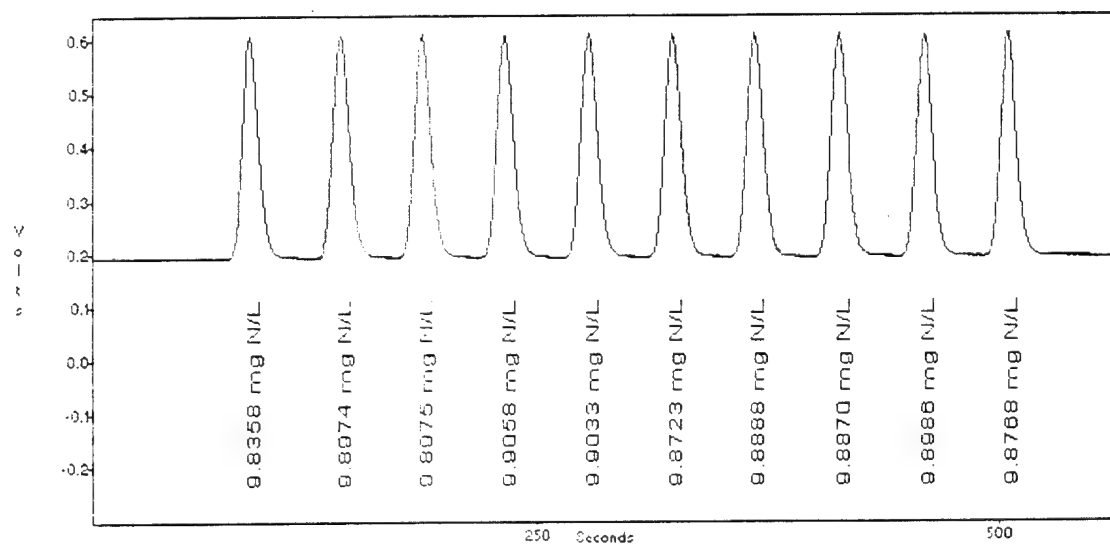
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME: Aug 22, 1994 13:54:02  
 DATA FILENAME: C:\OMNION\DATA\1010762E\082294M1.FDT  
 METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

Figure 3. Precision

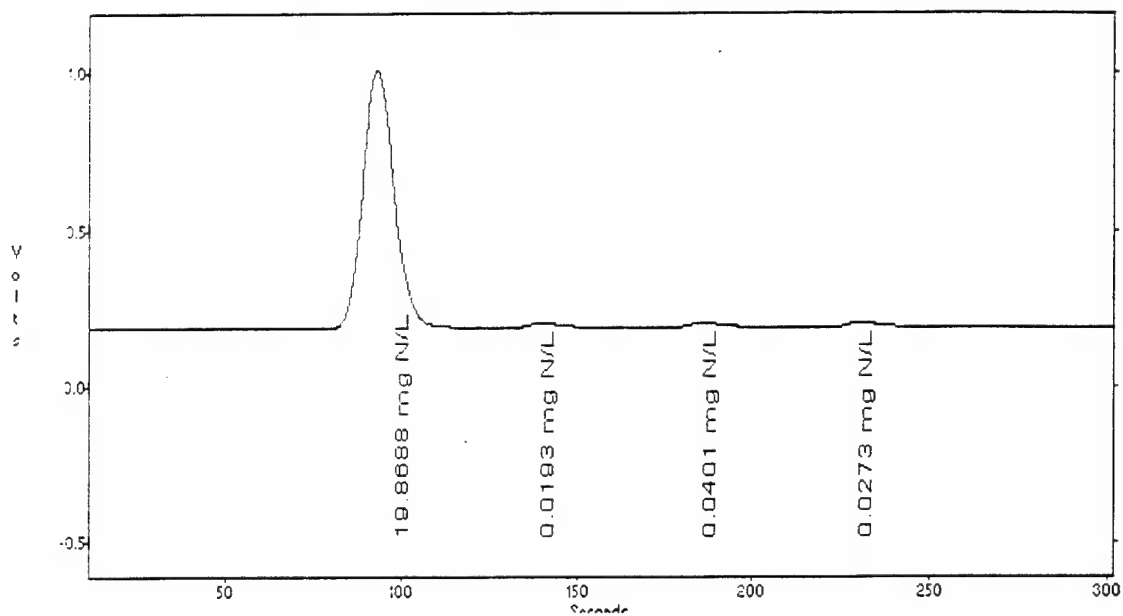


Precision = 0.211 % RSD

ACQ. TIME: Aug 22, 1994 14:21:28  
 DATA FILENAME: C:\OMNION\DATA\1010762E\082294P1.FDT  
 METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET



Figure 4. Carryover



ACQ. TIME: Aug 22, 1994 14:32:42  
DATA FILENAME: CAOMNION\DATA\1010762E\082294R1.FDT  
METHOD FILENAME: CAOMNION\METHODS\1010762D\1010762D.MET



**QuikChem METHOD 10-107-06-2-E**

**DETERMINATION OF TOTAL KJELDAHL NITROGEN BY**  
**FLOW INJECTION ANALYSIS COLORIMETRY**  
**(BLOCK DIGESTOR METHOD)**

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS  
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## QuikChem Method 10-107-06-2-E


# Total Kjeldahl Nitrogen in Waters

0.1 to 5.0 mg N/L

### -- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

### -- Interferences --

- 
1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H<sub>2</sub>SO<sub>4</sub> in the diluted digestion sample with no change in signal intensity.
  2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

### -- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers



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## DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

### 1. SCOPE AND APPLICATION

- 1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.
- 1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.
- 1.3. The applicable range is 0.1 to 5 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.
- 1.4. Samples containing particulates should be filtered or homogenized.

### 2. SUMMARY OF METHOD

- 2.1. The sample is heated in the presence of sulfuric acid,  $\text{H}_2\text{SO}_4$ , for two and one half hours. The residue is cooled, diluted with water and analyzed for ammonia. This digested sample may also be used for phosphorus determination.
- 2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$ , under the conditions of the digestion described.
- 2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.
- 2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.
- 2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.



### 3. DEFINITIONS

- 3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.
- 3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.
- 3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.
- 3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of



calibration standards. It is used to check laboratory performance with externally prepared test materials.

- 3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

#### 4. INTERFERENCES

- 4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H<sub>2</sub>SO<sub>4</sub> in the diluted digestion sample with no change in signal intensity.
- 4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.
- 4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

#### 5. SAFETY

- 5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.
- 5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.
- 5.3.1. Mercury (Reagents 1 and 2)
- 5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)
- 5.3.3. Sodium Nitroprusside (Reagent 4)



## 6. EQUIPMENT AND SUPPLIES

- 6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.
- 6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
  - 6.3.1. Sampler
  - 6.3.2. Multichannel proportioning pump
  - 6.3.3. Reaction unit or manifold
  - 6.3.4. Colorimetric detector
  - 6.3.5. Data system
- 6.4. Special apparatus
  - 6.4.1. Heating Unit
  - 6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)
  - 6.4.3. 5 mL and 20 mL repipet dispensers
  - 6.4.4. Vortex mixer

## 7. REAGENTS AND STANDARDS

### 7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

#### **Degassing with Helium**

To prevent bubble formation, the water carrier is degassed with helium. Use He at 20 lb/in<sup>2</sup> through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degassed.



#### Reagent 1.      Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 40.0 mL water and **10 mL** concentrated **sulfuric acid** ( $\text{H}_2\text{SO}_4$ ). Then add **8.0 g red mercuric oxide** ( $\text{HgO}$ ). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

#### Reagent 2.      Digestion Solution

In a 1 L volumetric flask, add **133.0 g potassium sulfate** ( $\text{K}_2\text{SO}_4$ ) and **200 mL concentrated sulfuric acid** ( $\text{H}_2\text{SO}_4$ ) to approximately **700 mL water**. Add **25.0 mL Reagent 1**. Dilute to the mark with water and invert to mix. Prepare fresh monthly.

#### Reagent 3.      Buffer

**By Volume:** In a 1 L volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** ( $\text{NaOH}$ ), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

**By Weight:** To a tared 1 L container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** ( $\text{NaOH}$ ). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

#### Reagent 4.      Salicylate Nitroprusside

**By Volume:** In a 1 L volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt,  $\text{C}_6\text{H}_4(\text{OH})(\text{COO})\text{Na}$ ], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ ] in about **800 mL water**. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

**By Weight:** To a tared 1 L dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt,  $\text{C}_6\text{H}_4(\text{OH})(\text{COO})\text{Na}$ ], **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ ] and **908 g water**. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.



**Reagent 5. Hypochlorite Solution**

**By Volume:** In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

**By Weight:** To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

**Reagent 6. Diluent 5.0% (V/V) Sulfuric Acid**

**NOTE:** Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

**By Volume:** In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

**By Weight:** To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2** (Digestion Solution). Invert to mix.



## 7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

### Digested Standards

**NOTE:** Working standards prepared in DI water are digested per the procedure in section 8.

#### Standard 1: Stock Standard 250 mg N/L

In a 1 L volumetric flask dissolve **0.9540 g ammonium chloride** ( $\text{NH}_4\text{Cl}$ ) that has been dried for two hours at  $110^\circ\text{C}$  in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500 (use 1.18g).

#### Standard 2. Working Stock Standard 5.0 mg N/L

**By Volume:** In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

**By Weight:** To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

#### By Volume

Volume (mL) of <b>Standard 2</b> diluted to 100 mL with DI water	100	40	20	10	5	2	0
--	-----	----	----	----	---	---	---

#### By Weight

Weight (g) of <b>Standard 2</b> diluted to final weight ( ~ 250 g) divide by <b>factor</b> below with DI water.	250.0	100	50	25	12.5	5	0
<b>Division Factor</b>	1.00	0.40	0.20	0.10	0.05	0.02	0
Divide exact weight of the standard by this <b>factor</b> to give final weight							



## Non-Digested Standards

### Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

**By Volume:** In a 1 L volumetric flask containing approximately 600 mL water, add 250 mL Reagent 2 (Digestion Solution). Dilute to the mark and invert to mix.

**By Weight:** To a tared 1 L container, add 760 g water and 250 mL Reagent 2 (Digestion Solution). Invert to mix.

### Standard 4. High Standard in Digestion Matrix (5.00 mg N/L)

**By Volume:** In a 1 L volumetric flask containing approximately 600 mL water, add 250 mL Reagent 2 (Digestion Solution). Add 20 mL of Standard 1 (250 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

**By Weight:** To a tared 1 L container, add 740 g water and 250 mL Reagent 2 (Digestion Solution). Add 20 g of Standard 1 (250 mg N/L) and shake to mix.

**Note:** Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg N/L for a 5.00 mg N/L non-digested standard. If non-digested standards are used to calibrate, the "labeled" concentrations should be entered in the data system.

Labeled non-digested standard concentration =  $\frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$

### Preparation of Non-digested Working Standards

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

#### By Volume

Volume (mL) of Standard 4 diluted to 100 mL with Standard 3	100	40	20	10	5	2	0
---	-----	----	----	----	---	---	---

#### By Weight

Weight (g) of Standard 4 diluted to final weight (~250 g) divide by factor below with Standard 3.	250.0	100	50	25	12.5	5	0
Division Factor	1.00	0.40	0.20	0.10	0.05	0.02	0
Divide exact weight of the standard by this factor to give final weight							



## **8. SAMPLE COLLECTION, PRESERVATION AND STORAGE**

- 8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.
- 8.2 Samples should be preserved to  $\text{pH} < 2$  and cooled to  $4^{\circ}\text{C}$  at the time of collection.
- 8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at  $4^{\circ}\text{C}$  and may be held for up to 28 days.

## **9. QUALITY CONTROL**

- 9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

### **9.2 INITIAL DEMONSTRATION OF PERFORMANCE**

- 9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.
- 9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being  $> 10\text{X MDL}$ . If any determined concentration exceeds the known values by  $\pm 10\%$ , linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.
- 9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within  $\pm 10\%$  of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.



- 9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where,  $t$  = Student's  $t$  value for a 99% confidence level and a standard deviation estimate with  $n-1$  degrees of freedom [ $t = 3.14$  for seven replicates,  $t = 2.528$  for twenty one replicates].

$S$  = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

### 9.3. ASSESSING LABORATORY PERFORMANCE

- 9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.
- 9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.3.3. The laboratory must use LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery ( $\bar{X}$ ) and the standard deviation ( $S$ ) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \bar{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation ( $S$ ) data should be used to establish an on-going



precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

- 9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

#### 9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

- 9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.
- 9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

Where,

R = percent recovery

$C_s$  = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

- 9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.



- 9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

## **10. CALIBRATION AND STANDARDIZATION**

- 10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).
- 10.4. Calibrate the instrument as description in section 11.
- 10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.
- 10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed  $\pm 10\%$  of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

## **11. PROCEDURE**

### **11.1. DIGESTION PROCEDURE**

**NOTE:** Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

- 11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.
- 11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)
- 11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar



granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

- 11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digester for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.
- 11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.
- 11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.
- 11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.
- 11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

## 11.2. SYSTEM START-UP PROCEDURE

- 11.2.1. Prepare reagent and standards as described in section 7.
- 11.2.2. Set up manifold as shown in section 17.1.
- 11.2.3. Input peak timing and integration window parameters as specified in section 17.
- 11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.
- 11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.
- 11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).



## 11.4. SYSTEM NOTES

- 11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.
- 11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.
- 11.4.3. If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.
- 11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.
- 11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.
- 11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.
- 11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:
  - A. Place transmission lines in water and pump to clear reagents (2-5 minutes).
  - B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.
  - C. Place all transmission lines in water and pump for several minutes.
  - D. Resume pumping reagents.

## 12. DATA ANALYSIS AND CALCULATIONS

- 12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.



- 12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3. Report results in mg N/L.

### **13. METHOD PERFORMANCE**

- 13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

### **14. POLLUTION PREVENTION**

- 14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.
- 14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

### **15. WASTE MANAGEMENT**

- 15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the



"Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

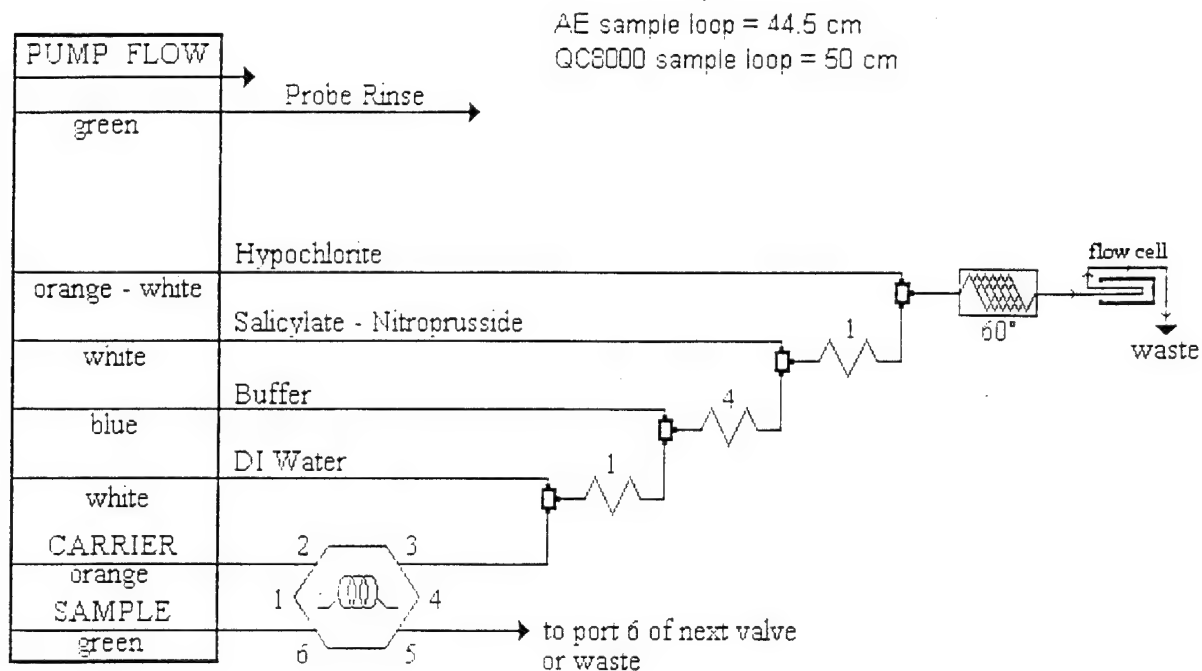
## 16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.



## 17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

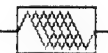
### 17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is 70 cm of tubing on a 1 inch coil support

4 is 255 cm of tubing on a 4 inch coil support

**Apparatus:** Standard valve, flow cell, and detector head modules are used. The  shows 650 cm of heated tubing. All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc



## 17.2. DATA SYSTEM PARAMETERS FOR QUIKCHEM AE

Sample throughput: 90 samples/hour; 60 s/sample  
Pump speed: 35  
Cycle Period: 45 s

Inject to start of peak period: 38 s

Presentation. Data Window

Top Scale Response: 0.25 abs

Bottom Scale Response: 0.00 abs

Segment/Boundaries: A: 5.00 mg N/L

C: 1 mg N/L

G: 0.00 mg N/L

Series 4000/System IV Settings: Gain = 570 x 1



# 17.3. QUIKCHEM AE SUPPORT DATA

1a. IKN Calibration (92062501)

Chord(s) J

Std	mg N/L
A	5.00
B	2.00
C	1.00
D	0.50
E	0.25
F	0.10
G	0.00

Cal Ref: 92062501  
Method: IKN-F

Calibration Statistics Report

06/25/92 01:43 pm

Channel: IKN

Correlation Coefficients

Seq	Std	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-C	1.0000	0.9992	0.9997	1.0000	0.9999	1.0000
2	C-G	0.9906	0.9384	0.9780	0.9978	0.9951	0.9992

Percent Standard Deviation in Slope

	1	2	3	4	5	6
1 A-C	0.3	2.8	1.3	0.5	0.0	1.1
2 C-G	2.2	20.3	2.6	0.8	3.7	51.6

Duke/Dave AE Calibration Report for Calibration 92062501

Method: IKN

This calibration was done on 06/25/92 at 10:25 pm

This report prepared on 06/25/92 at 01:43 pm

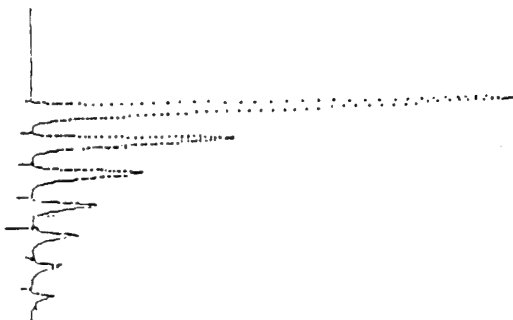
Standard	Analyte	Units mg N/L	Average Concentrations			Baseline Corrected Average Absorbance
			Known	Determined	% Residual	
Standard A, IKN	Analyte	Units mg N/L	5.000	5.003	-0.05	0.1715
Standard B, IKN	Analyte	Units mg N/L	2.000	1.989	0.54	0.0711
Standard C, IKN	Analyte	Units mg N/L	1.000	1.001	-0.26	0.0381
Standard D, IKN	Analyte	Units mg N/L	0.500	0.496	0.78	0.0229
Standard E, IKN	Analyte	Units mg N/L	0.250	0.242	3.27	0.0137
Standard F, IKN	Analyte	Units mg N/L	0.100	0.102	-1.77	0.0072
Standard G, IKN	Analyte	Units mg N/L	0.000	0.006	100.00	0.0061

End of Calibration Report for Calibration 92062501



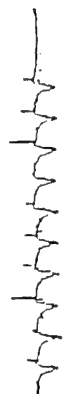
Calibration 06/25/1992, 12:17 PM

- 1. Standard A (1)
- 2. Standard B (1)
- 3. Standard C (1)
- 4. Standard D (1)
- 5. Standard E (1)
- 6. Standard F (1)
- 7. Standard G (1)



Back 1 (File: 92062502) 06/25/1992, 12:23 PM

- 101. Blank 4.84 H2SO4 (1)
- 102. Blank 4.84 H2SO4 (1)
- 103. Blank 4.84 H2SO4 (1)
- 104. Blank 4.84 H2SO4 (1)
- 105. Blank 4.84 H2SO4 (1)
- 106. Blank 4.84 H2SO4 (1)
- 107. Blank 4.84 H2SO4 (1)
- 108. Blank 4.84 H2SO4 (1)
- 109. Blank 4.84 H2SO4 (1)
- 110. Blank 4.84 H2SO4 (1)



Cup	Sample ID	1 TKH mg H/L
101	Blank 4.84 H2SO4	0.013<
102	Blank 4.84 H2SO4	0.012<
103	Blank 4.84 H2SO4	0.009<
104	Blank 4.84 H2SO4	0.003<
105	Blank 4.84 H2SO4	0.049<
106	Blank 4.84 H2SO4	-0.007<
107	Blank 4.84 H2SO4	0.010<
108	Blank 4.84 H2SO4	0.009<
109	Blank 4.84 H2SO4	0.009<
110	Blank 4.84 H2SO4	-0.013<

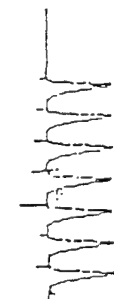
ENDL Determination

mean = 0.009  
s = 0.016  
4.65 (a) = 0.076 mg H/L

----- End of Report for Tray 92062502.RS -----

File: 92062504 06/25/1992, 12:41 PM

- 101. 0.5 mg H/L (1)
- 102. 0.5 mg H/L (1)
- 103. 0.5 mg H/L (1)
- 104. 0.5 mg H/L (1)
- 105. 0.5 mg H/L (1)
- 106. 0.5 mg H/L (1)
- 107. 0.5 mg H/L (1)



Cup	Sample ID	1 TKH mg H/L
101	0.5 mg H/L	0.409
102	0.5 mg H/L	0.406
103	0.5 mg H/L	0.400
104	0.5 mg H/L	0.491
105	0.5 mg H/L	0.470
106	0.5 mg H/L	0.492
107	0.5 mg H/L	0.490

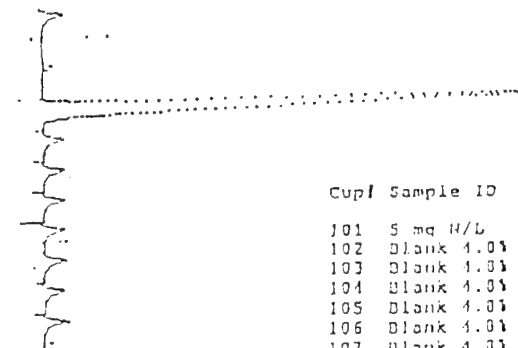
HDL Study

mean = 0.407  
s = 0.000  
(3.14) s = 0.024

----- End of Report for Tray 92062504.RS -----

Back 1 (File: 92062506) 06/25/1992, 12:54 PM

- 101. 5 mg H/L (1)
- 102. Blank 4.84 H2SO4 (1)
- 103. Blank 4.84 H2SO4 (1)
- 104. Blank 4.84 H2SO4 (1)
- 105. Blank 4.84 H2SO4 (1)
- 106. Blank 4.84 H2SO4 (1)
- 107. Blank 4.84 H2SO4 (1)
- 108. Blank 4.84 H2SO4 (1)



Cup	Sample ID	1 TKH mg H/L
101	5 mg H/L	4.052
102	Blank 4.84 H2SO4	0.003<
103	Blank 4.84 H2SO4	0.002<
104	Blank 4.84 H2SO4	0.001<
105	Blank 4.84 H2SO4	-0.001<
106	Blank 4.84 H2SO4	0.009<
107	Blank 4.84 H2SO4	0.004<
108	Blank 4.84 H2SO4	-0.009<

Carry-over Study

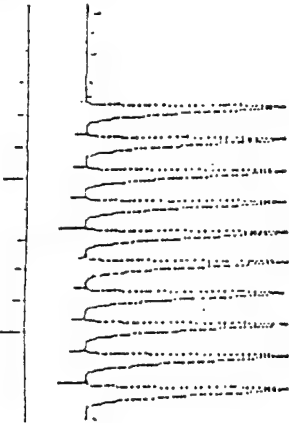
mean = 0.001  
s = 0.005  
95% CI (-0.004 to 0.006)  
passes

----- End of Report for Tray 92062506.RS -----



1 (Ref: 92062507) 06/23/1992, 01:04 pm

0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)  
0.1 mg H/L (1)



Cup/ Sample ID

		1 TKN mg H/L
101	2.0 mg H/L	1.923
102	2.0 mg H/L	1.926
103	2.0 mg H/L	1.925
104	2.0 mg H/L	1.926
105	2.0 mg H/L	1.947
106	2.0 mg H/L	1.912
107	2.0 mg H/L	1.903
108	2.0 mg H/L	1.933
109	2.0 mg H/L	1.955
110	2.0 mg H/L	1.963

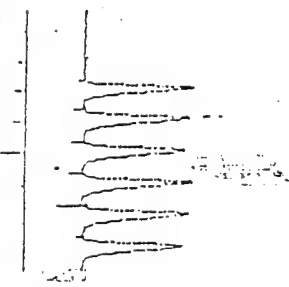
Precision at Midscale

mean = 1.929  
s = 0.023  
RSD = 1.18

----- End of Report for Tray 92062507.RS -----

1 (Ref: 92062503) 06/23/1992, 12:35 pm

0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)  
0.1 mg H/L 4.0% H2SO4 (1)



Cup/ Sample ID

		1 TKN mg H/L
101	1.0 mg H/L 4.0% H2SO4	0.966
102	1.0 mg H/L 4.0% H2SO4	0.960
103	1.0 mg H/L 4.0% H2SO4	0.994
104	1.0 mg H/L 4.0% H2SO4	0.975
105	1.0 mg H/L 4.0% H2SO4	0.925
106	1.0 mg H/L 4.0% H2SO4	0.900

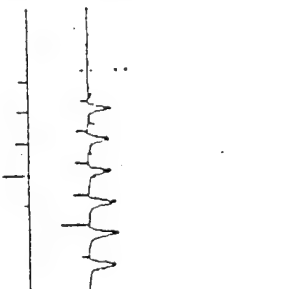
Acid Effect

mean (4.0%) = 0.971  
mean (4.0%) = 0.943  
mean (4.0%) = 0.891

----- End of Report for Tray 92062503.RS -----

1 (Ref: 92062508) 06/23/1992, 01:14 pm

0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)  
0.1 mg Ca/L (1)



Cup/ Sample ID

		1 TKN mg H/L
101	20 mg Ca/L	-0.013<
102	20 mg Ca/L	0.005<
103	20 mg Ca/L	0.020<
104	100 mg Ca/L	0.052<
105	100 mg Ca/L	0.050<
106	100 mg Ca/L	0.060<

Calcium Interference Study

mean (20 mg/L) = 0.004  
mean (100 mg/L) = 0.059  
<MDL at 100 mg/L

----- End of Report for Tray 92062508.RS -----



#### 17.4. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput: 90 samples/hour; 60 s/sample  
Pump speed: 35  
Cycle Period: 45 s

##### Analyte data:

Peak Base Width: 31 s  
% Width Tolerance: 100  
Threshold: 25000  
Inject to Peak Start: 42 s  
Chemistry: Direct

##### Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

##### Sampler Timing:

Min. Probe in Wash Period: 14 s  
Probe in Sample Period: 20 s

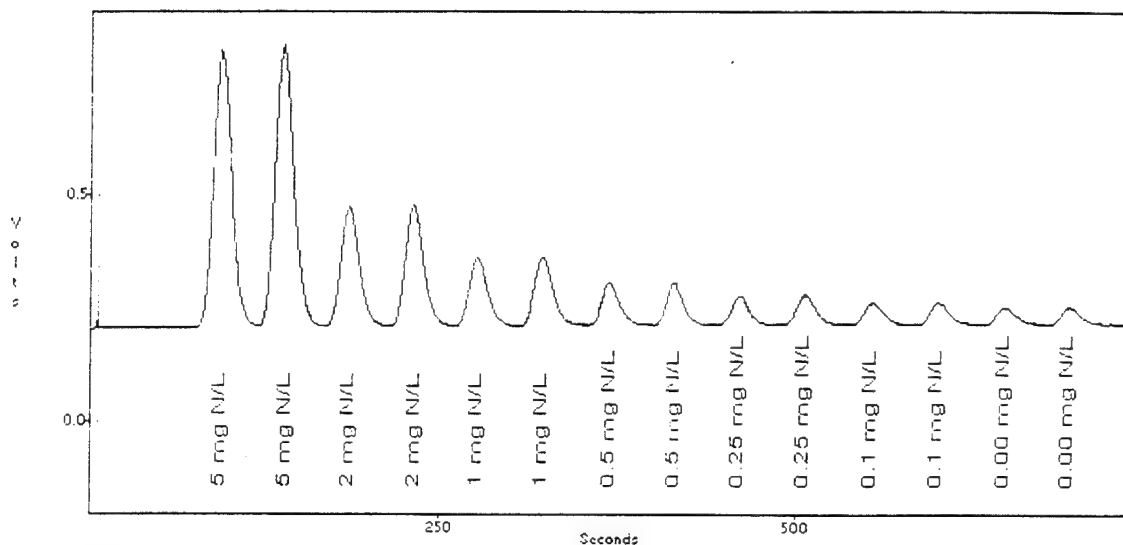
##### Valve Timing:

Load Period: 20 s  
Inject Period: 25 s  
Load Time: 0.0 s



## 17.5 QUIKCHEM 8000 SUPPORT DATA

Figure 1. Calibration Graph and Statistics for Total Kjeldahl Nitrogen



ACQ. TIME: Aug 15, 1994 15:00:17  
 DATA FILENAME: C:\OMNION\DATA\1010762E\081594C1.FDT  
 METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8778128	5	5.000	8851078	8705178	1003166.9	1.2	0.0
2	3809886	2	2.002	3832259	3787513	31640.2	0.8	-0.1
3	2159521	1	1.006	2157189	2161852	3296.9	0.2	-0.6
4	1326319	0.5	0.5035	1335357	1317280	12782.8	1.0	-0.7
5	896683	0.25	0.2445	890758	902609	8380.0	0.9	2.2
6	643806	0.1	0.108	648879	638732	7174.7	1.1	8.0
7	498016	0	0	5071570	488876	12926.6	2.6	---

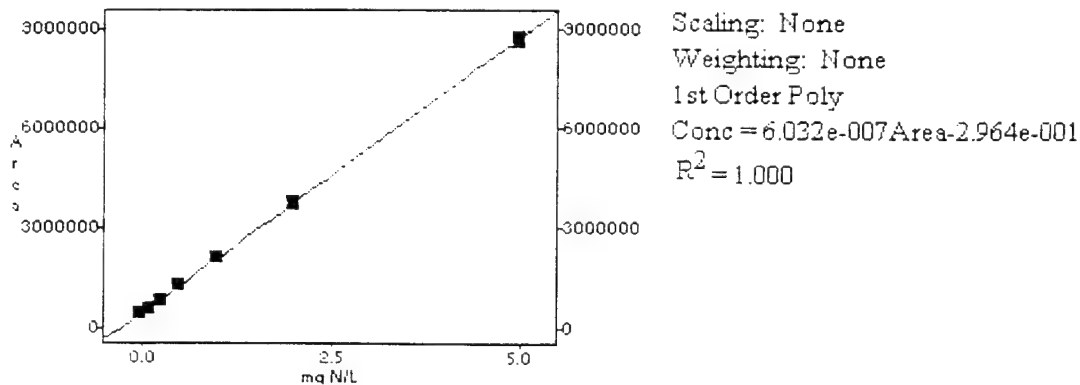
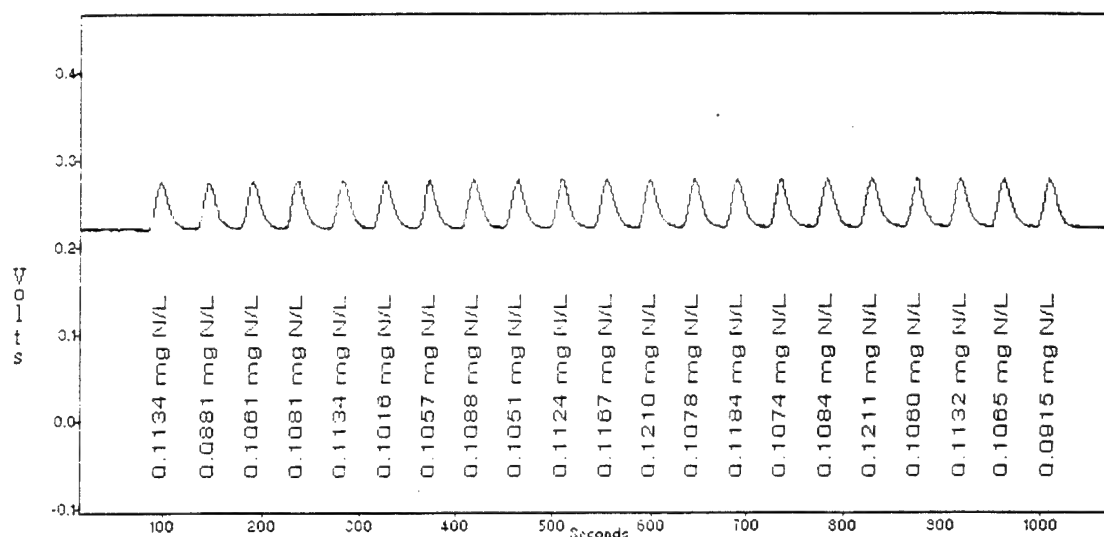




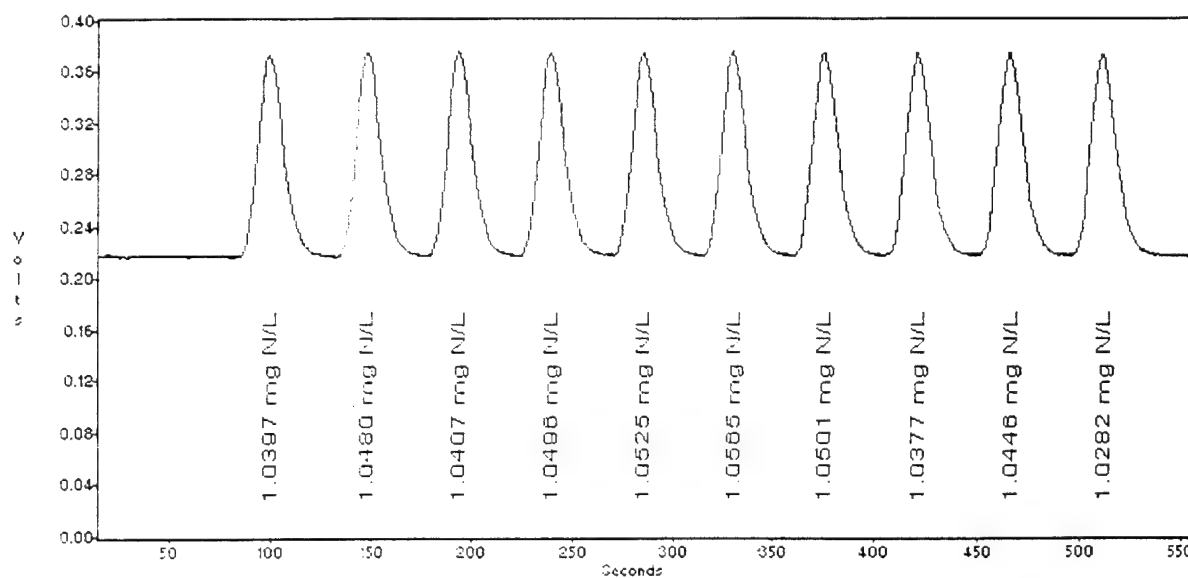
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME: Aug 18, 1994 8:52:31  
 DATA FILENAME: C:\OMNION\DATA\1010762E\081894M1.FDT  
 METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

Figure 3. Precision

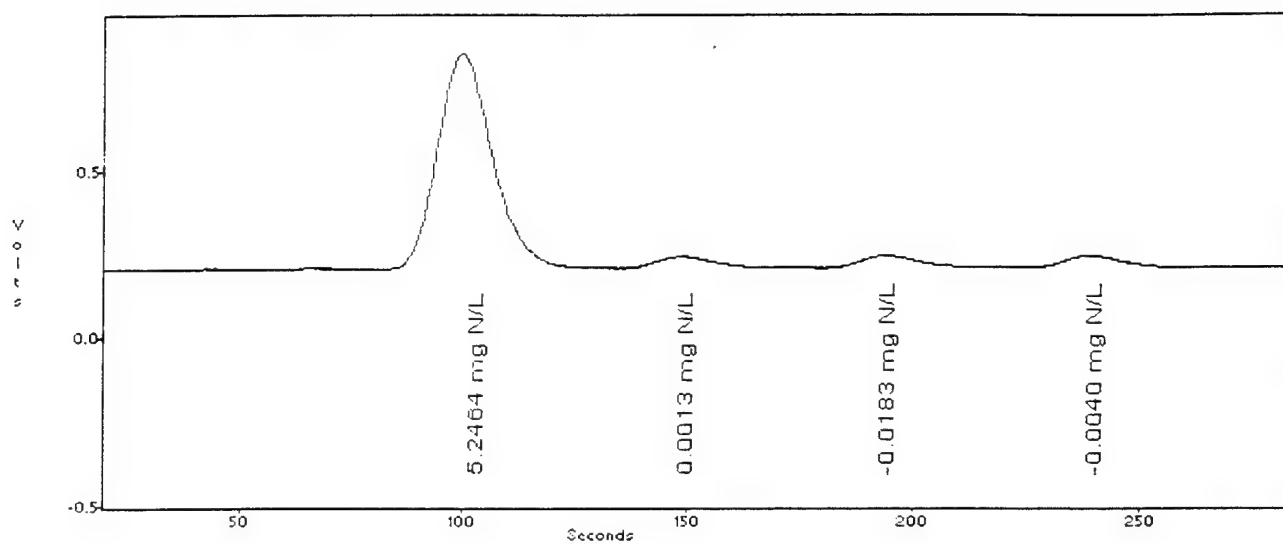


Precision = 0.796 % RSD

ACQ. TIME: Aug 18, 1994 15:50:24  
 DATA FILENAME: C:\OMNION\DATA\1010762E\081594P1.FDT  
 METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met



Figure 4. Carryover



Carryover passed

ACQ. TIME:

Aug 15, 1994 16:01:01

DATA FILENAME:

C:\NOMNION\DATA\1010762E\081594R1.FDT

METHOD FILENAME:

C:\NOMNION\METHODS\1010762E\1010762E.met



# Technicon™ AutoAnalyzer III™

INDUSTRIAL METHOD No. 334-74W/B<sup>+</sup>

RELEASED: JANUARY, 1976/REVISED: MARCH 1977

## INDIVIDUAL/SIMULTANEOUS\* DETERMINATION OF NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

RANGE: Nitrogen 1-50 mg/l; 20-1000 mg/l  
Phosphorus 1-50 mg/l; 20-1000 mg/l  
BD-20/BD-40 (DIALYZER)

### GENERAL DESCRIPTION

#### NITROGEN

The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at a pH of 12.8-13.0. The ammonia-salicylate complex is read at 660 nm.

#### PHOSPHORUS

The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm.

The acid digest samples are prepared by digestion with the Technicon BD-40 or BD-20 Block Digestor. Refer to Manual No. TA4-0323-11 for sample preparation.

### PERFORMANCE AT 40 SAMPLES PER HOUR

#### MANUALLY PREPARED STANDARDS

##### NITROGEN

	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 1.00 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.6%	at 500 mg/l ±0.4%
Detection Limit	1.0 mg/l	20 mg/l

##### PHOSPHORUS

	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 0.60 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.5%	at 500 mg/l ±0.6%
Detection Limit	1.0 mg/l	20 mg/l

\*See Operating Note 7.



TECHNICON INDUSTRIAL SYSTEMS / TARRYTOWN, N.Y. 10591  
A DIVISION OF TECHNICON INSTRUMENTS CORPORATION

PRINTING CODE:  
A/11/a/1  
4732-1-6/R3-7-1.5



## REAGENTS

Unless otherwise specified, all reagents should be of ACS quality or equivalent.

### GENERAL REAGENTS

#### TRITON X-100 SOLUTION (50% in Methanol)

Triton X-100**	
(Technicon No. T21-0188)	50 ml
Methanol ( $\text{CH}_3\text{OH}$ )	50 ml

##### Preparation:

Add 50 ml of Triton X-100 to 50 ml of methanol and mix thoroughly.

#### SYSTEM WASH WATER SOLUTION

(For System Shut-Down and Start-Up Only)

Triton X-100 Solution	1.0 ml
Distilled Water	1000 ml

##### Preparation:

Add 1.0 ml of Triton X-100 solution to one liter of distilled water and mix.

#### SAMPLER IV WASH RECEPTACLE SOLUTION

Distilled Water

Note: This reagent contains *no* wetting agent.

### NITROGEN REAGENTS

#### STOCK SODIUM HYDROXIDE SOLUTION, 20%

Sodium Hydroxide Solution,	
50% w/w	400 g
Distilled Water, q.s.	1000 ml

##### Preparation:

To 600 ml of distilled water, add 400 g of sodium hydroxide solution, 50% w/w. Cool to room temperature and dilute to one liter with distilled water.

#### STOCK SODIUM POTASSIUM TARTRATE SOLUTION, 20%

Sodium Potassium Tartrate	
( $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ )	200 g
Distilled Water, q.s.	1000 ml

##### Preparation:

Dissolve 200 g of sodium potassium tartrate in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly.

#### STOCK BUFFER SOLUTION 0.5M

Sodium Phosphate, Dibasic, crystal	
( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ )	134 g
[Sodium Phosphate, Dibasic,	
anhydrous ( $\text{Na}_2\text{HPO}_4$ )]	[71 g]
Sodium Hydroxide Solution,	
50% w/w	40 g
Distilled Water, q.s.	1000 ml

##### Preparation:

Dissolve 134 g of sodium phosphate, dibasic, crystal (or 71 g of sodium phosphate, dibasic, anhydrous) in about 800 ml of distilled water. Add 40 g of sodium hydroxide solution, 50% w/w, dilute to one liter with distilled water and mix thoroughly.

#### WORKING BUFFER SOLUTION

Stock Buffer Solution, 0.5M	200 ml
Stock Sodium Potassium Tartrate	
Solution, 20%	250 ml
Stock Sodium Hydroxide	
Solution, 20%	250 ml
Distilled Water, q.s.	1000 ml
Brij-35,*** 30% Solution	
(Technicon No. T21-0110)	1.0 ml

##### Preparation:

Combine the reagents in the stated order: add 250 ml of stock sodium potassium tartrate solution, 20%, to 200 ml of stock buffer solution, 0.5M, with swirling. Slowly, with swirling, add 250 ml of sodium hydroxide solution, 20%. Dilute to one liter with distilled water. add 1.0 ml of Brij-35, 30% solution, (20-25 drops) and mix thoroughly.

#### SULFURIC ACID/SODIUM CHLORIDE SOLUTION

Sulfuric Acid, 95-98%	
( $\text{H}_2\text{SO}_4$ )	7.5 ml
Sodium Chloride (NaCl)	100 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% Solution	1.0 ml

##### Preparation:

Dissolve 100 g of sodium chloride in about 600 ml of distilled water. Add 7.5 ml of sulfuric acid and dilute to one liter with distilled water. Add 1.0 ml of Brij-35 (about 20 drops) and mix thoroughly.

#### SODIUM SALICYLATE/SODIUM NITROPRUSSIDE SOLUTION

Sodium Salicylate ( $\text{NaC}_7\text{H}_5\text{O}_3$ )	150 g
Sodium Nitroprusside	
( $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ )	0.30 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% solution	1.0 ml

\*\*Trademark of Rohm and Haas Company.

\*\*\*Trademark of Atlas Chemical Industries, Inc.



#### Preparation:

Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 ml of distilled water. Filter through fast filter paper into a one liter volumetric flask and dilute to volume with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly. Store in a light-resistant container.

#### SODIUM HYPOCHLORITE SOLUTION, 0.315%

Sodium Hypochlorite	
Solution, 5.25%	6.0 ml
Distilled Water, q.s.	100 ml
Brij-35, 30% Solution	0.1 ml

#### Preparation:

Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with distilled water. Add 0.1 ml (2 drops) of Brij-35 and mix thoroughly. Prepare fresh daily. [Any commercial bleach solution (e.g. Clorox) containing 5.25% available chlorine is satisfactory.]

#### PHOSPHORUS REAGENTS

#### SULFURIC ACID SOLUTION, 4.0N

Sulfuric Acid, 95-98% ( $H_2SO_4$ )	111 ml
Distilled Water, q.s.	1000 ml
Triton X-100 Solution	1.0 ml

#### Preparation:

While swirling, cautiously add 111 ml of sulfuric acid to about 600 ml of distilled water. Cool to room temperature and dilute to one liter with distilled water. Add 1.0 ml of Triton X-100 solution and mix thoroughly.

#### SODIUM CHLORIDE SOLUTION, 0.25%

Sodium Chloride (NaCl)	2.5 g
Distilled Water, q.s.	1000 ml
Aerosol-22****	5.0

#### Preparation:

Dissolve 2.5 g of sodium chloride in about 600 ml of distilled water. Dilute to one liter with distilled water. Add 5.0 ml of Aerosol-22 and mix thoroughly.

#### MOLYBDATE/ANTIMONY SOLUTION

Ammonium Molybdate	
$[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$	10.0 g
Antimony Potassium Tartrate	
$[K(SbO)C_4H_4O_6 \cdot 1/2H_2O]$	0.15 g
Sulfuric Acid, 95-98% ( $H_2SO_4$ )	60 ml
Distilled Water, q.s.	1000 ml

#### Preparation:

Dissolve 10.0 g of ammonium molybdate and 0.15 g of antimony potassium tartrate in about 800 ml of distilled water. While swirling, cautiously add 60 ml

of sulfuric acid. Cool to room temperature, dilute to one liter with distilled water and mix thoroughly. Transfer to a light-resistant container. This solution is stable for about one month.

#### ASCORBIC ACID SOLUTION, 1.0%

Ascorbic Acid ( $C_6H_8O_6$ )	— OR —	2.0 g
Araboascorbic Acid ( $C_6H_8O_6$ )		
Distilled Water, q.s.		200 ml

#### Preparation:

Dissolve 2.0 g of ascorbic acid or araboascorbic acid in about 150 ml of distilled water. Dilute to 200 ml with distilled water and mix thoroughly. Transfer to a light-resistant container. If kept refrigerated and tightly stoppered when not in use, this solution is stable for at least two days.

#### OPERATING NOTES

##### 1. Start-Up

- Check the level of all reagents to ensure an adequate supply.
- Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers.
- When reagents have been pumping for at least five minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate for 10 minutes.

NOTE: If a precipitate appears after the addition of salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid is caused by a low pH. Before restarting the system, check the concentration of the sulfuric acid solution and/or the working buffer solution.

- To prevent precipitation of salicylic acid in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen colorimeter TO WASTE tube separate from all other lines or keep tap water flowing in the waste tray.

##### 2. Shut-Down

- Remove the salicylate and molybdate/antimony lines from their containers and allow them to pump air. When the air bubbles enter the analytical system, place all reagent lines (excluding the Sampler IV Wash Receptacle Solution line) in the System Wash Water Solution.
- After 15 minutes, stop the proportioning pump and remove the platen.

\*\*\*\*Trademark of American Cyanamid Company.



### 3. System Operation

- a. Be sure the plastic cover of the analytical cartridge is in place when operating the system.
- b. At STD CAL settings of 6.00 or more, the system may be operated in the DAMP 1 position, if necessary.

### 4. Manifold Connections

To avoid the possibility of airborne contamination, the air lines of the nitrogen channel should be attached to an air scrubber containing dilute sulfuric acid (10% v/v).

### 5. Reagent Background Color

- a. Place all lines in the system wash water container and start the proportioning pump. After making the necessary adjustments on the colorimeters set the STD CAL control of the nitrogen colorimeter to 1.00 and the STD CAL control of the phosphorus colorimeter to 2.90. Adjust the water baseline on both colorimeters to zero with the BLANK control.
- b. Following the start-up procedure, place all reagent lines in the proper order in their respective containers and allow the system to equilibrate.
- c. The reading of the reagents compared to distilled water should not be more than 14 units (0.140 absorbance) for the nitrogen channel and not more than 5 units (0.25 absorbance) for the phosphorus channel. If the absorbance of either channel is much higher than the above values, one or more of the reagents or the water used to make up the reagents is probably contaminated.

### 6. Concentration Ranges

- a. All concentration ranges refer to the concentration of components in the digestion tube after diluting to volume with distilled water.
- b. **Nitrogen Channel**
  1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the flowcell and the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 1 and flow diagram).
  2. For any one manifold configuration, an approximate five-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 1.00 or higher.

### 2. Phosphorus Channel

1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure and flow diagram).
2. For any one manifold configuration, an approximate three-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 2.00 or higher.

### 7. Manifold Configurations

#### a. Individual Determination of N or P

When N or P is being determined individually, the PT fitting is omitted and the sample line is attached directly to the sample probe of the Sampler IV.

#### b. Simultaneous Determination of N and P

When N and P are being determined simultaneously, both initial sample lines are connected to a PT stream-splitter fitting which is in turn connected to the sample probe on the Sampler IV.

### 8. Sample Probe and PT Stream-Splitter

Because stainless steel is susceptible to attack by sulfuric acid solutions, this method utilizes special Kel-F sample probe (Technicon No. 17 0745) and a special PT stream-splitter with platinum nipples (Technicon No. 116-B331).

### 9. Phosphorus Channel (only)

#### a. Cleansing Procedure

Before initially operating the system, the following procedure should be performed to cleanse the system. Once a week thereafter, this procedure should be repeated during system start-up.

With the exception of the ascorbic acid and molybdate/antimony lines, place all phosphorus reagent lines into their respective containers. Start the proportioning pump and allow five minute pumping time. Place both the ascorbic acid and molybdate/antimony lines in sodium hydroxide solution, 20% for five minutes, then into hydrogen peroxide, 50% for five minutes, then into distilled water. After five minutes follow the start-up procedure (Operating Note 1) and allow the system to equilibrate.



b. **Conditioning Procedure**

After the initial cleansing of the system is performed, condition the phosphorus channel as described below. Once this channel has been conditioned, there is no need to repeat the procedure; only the cleansing procedure need be performed once each week during start-up.

Following the Start-Up procedure (Operating Note #1), place all reagent lines for phosphorus in their respective containers and allow the system to equilibrate. Place three sample cups containing midscale standard solution on the Sampler IV tray (with a stop-pin at the third cup) and start the sampler. Aspirate the set of standards three times, allowing five minutes of wash between each set. After the Recorder traces the last standard peak, wait ten minutes and adjust the baseline tracing to zero using the BASELINE control.

10. **Crude Protein Determination — AOAC**

When this methodology is utilized to assay acid digestates for the determination of Crude Protein in Feeds by the official AOAC procedure, the following hardware changes **must** be incorporated into the system:

- a. **Sampler IV** — Sampler IV cam must be 40/hour with a sample-to-wash ratio of 2:1 (cam is included in the accessories and spares kit).

- b. **Analytical Cartridge** — dilution loop pump tubes **must** be of the following size:

**INITIAL SAMPLE DILUTION**

Sample Line	0.16 ml/min (Orn/Yel)
H <sub>2</sub> SO <sub>4</sub> /NaCl Line	1.20 ml/min (Yel/Yel)

**RESAMPLE DILUTION**

Resample Line	0.16 ml/min (Orn/Yel)
H <sub>2</sub> SO <sub>4</sub> /NaCl Line	0.80 ml/min (Red/Red)

- c. **Colorimeter** — must be equipped with 15 mm pathlength flowcell (1.5 or 2.0 mm ID).



MANIFOLD NO. 116-D531-01

6



# INDIVIDUAL/SIMULTANEOUS NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

PHOSPHORUS RANGES: FROM 1-50 mg/l  
TO 20-1000 mg/l  
MANIFOLD NO. 116-D541-01

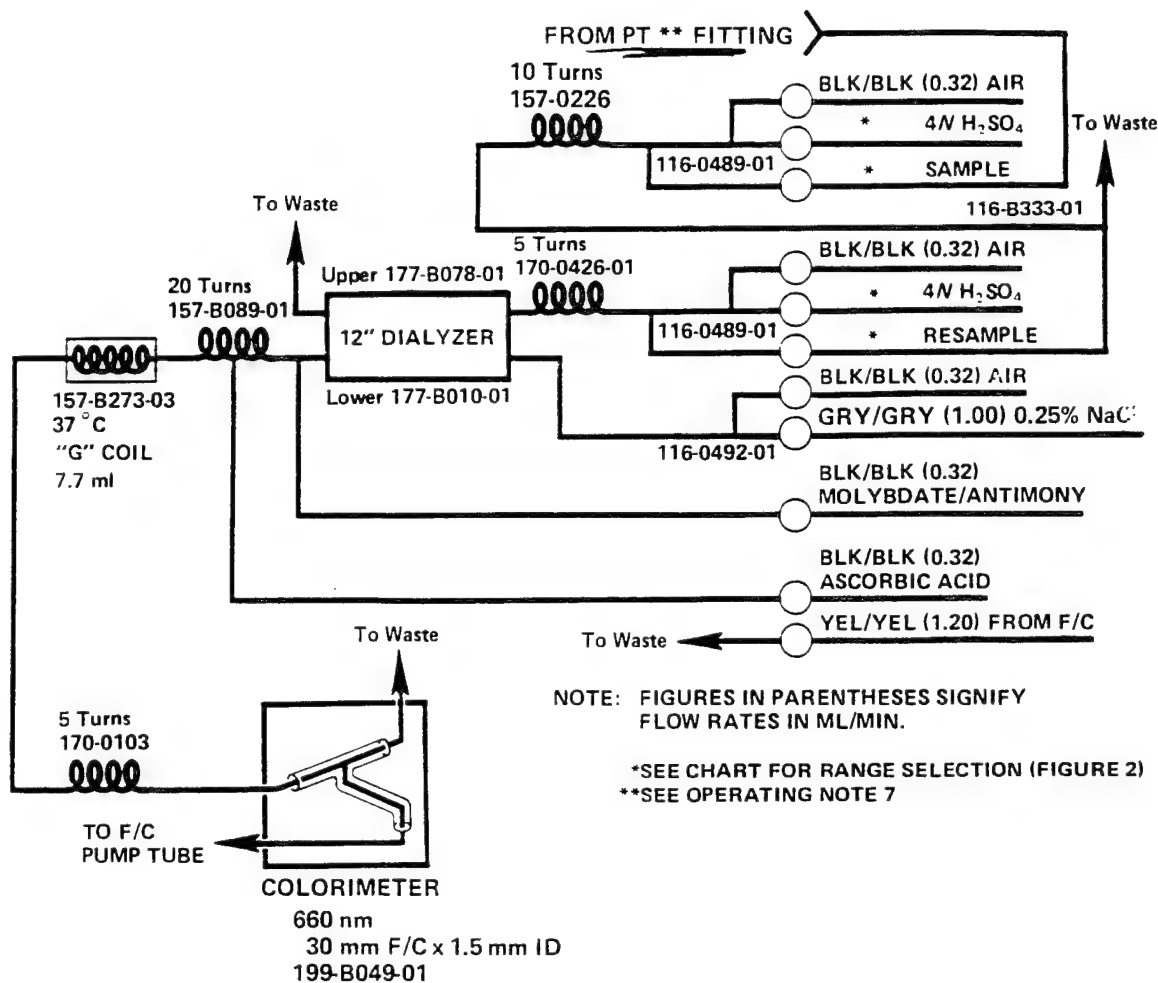


Figure 2. CONCENTRATION RANGES (PHOSPHORUS)

DILUTION LOOPS				APPROX. STD CAL SETTING	RANGE mg/l P (±10%)
INITIAL SAMPLE		RESAMPLE			
SAMPLE LINE	4N H <sub>2</sub> SO <sub>4</sub> LINE	RESAMPLE LINE	4N H <sub>2</sub> SO <sub>4</sub> LINE		
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.32 (Blk/Blk)	0.80 (Red/Red)	7.30 2.20	1-50 3-150
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	2-100 6-300
0.16 (Orn/Yel)	1.60 (Blu/Blu)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	6-300 20-1000



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# *AutoAnalyzer Application*

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Industrial Method - #376-75W/B

## **DIGESTION AND SAMPLE PREPARATION FOR THE ANALYSIS OF TOTAL KJELDAHL NITROGEN AND/OR TOTAL PHOSPHORUS IN WATER SAMPLES USING THE BRAN + LUEBBE BD-40 BLOCK DIGESTOR**

The following procedure is recommended for the analysis of nitrogen and/or phosphorus in water samples. Samples are digested using a Bran + Luebbe BD-40 Block Digestor and assayed using a Bran + Luebbe AutoAnalyzer II Continuous Flow Analytical System.

This procedure should be utilized in conjunction with the Operation Manual for the Block Digestor BD-20/40 (Bran + Luebbe Publication No. TA4-0323-11) and the methodology for Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests (Industrial Method No. 329-74W - Revised 11/78).

### **Introduction**

When analyzing water samples with the BD-40, the water present in the digestion tubes must be evaporated before heating the tubes at a temperature that is high enough to affect digestion. When water is added to sulfuric acid, the boiling point of the resultant mixture is considerably lower than that of sulfuric acid alone. If this mixture is placed in the block at a temperature significantly higher than its boiling point, the tube contents will bump out resulting in loss of sample, contamination of adjacent tubes, and most importantly, possible bodily injury.

The automatic (temperature-programmed) mode of the BD-40 is utilized to first evaporate the water and then to raise and maintain the block temperature high enough to affect digestion. At the end of the programmed cycle, the unit automatically shuts down.



Since the concentration levels of N and P in the samples encountered are frequently very low, accuracy and precision can be insured only if good analytical technique is employed during all steps of the procedure -- from sample preparation to assay of the digested samples. Every precaution should be observed to avoid contamination of sample tubes, pipets, reagents, spatulas, etc. The use of de-ionized, distilled water or its equivalent is required throughout the procedure. Contaminated water is the most frequently encountered source of difficulty in running this procedure.

### Concentration Ranges and Manifold Configuration

#### Samples

The choice of manifold configuration depends on the concentration of N and/or P in the sample.

Depending on the manifold configuration and STD CAL setting, the BD-40 related analytical cartridges can accommodate N and/or P in the ranges from 0.024 - 1.2 mg/l to 1.50 - 75 mg/l in the undigested sample.

The methodology (No. 329-74W) gives three configurations for the nitrogen and phosphorus cartridges and the concentration ranges for each of the configurations. Each configuration can accommodate approximately a five-fold change in concentration by varying the STD CAL control from 100 to 700. The range represents the detection limit (2% of full scale) and the full scale concentration for a particular STD CAL setting.

Samples containing higher levels of nitrogen or phosphorus should be diluted with distilled de-ionized water prior to digestion.

Referring back to Figures 1 and 2 of Method 329-74W: Because of the possibility of contamination, it is recommended that configuration #1 be used only for samples containing 15 mg N or P/l or less; all samples greater than 15 mg/l should be assayed on configuration #2 or #3. The choice of cartridge configuration is best illustrated by example.

Consider a group of samples containing 1 - 5 mg N/l and 5 - 50 mg P/l. The configuration of choice for nitrogen would be #2 adjusted with the STD CAL control to 5 mg/l full scale deflection. This would yield peaks ranging from 20% to full scale. The choice for phosphorus would be configuration #3 adjusted for a full scale deflection of 50 mg/l. This would yield peaks ranging from 10% to full scale.



Occasionally, the range of N or P in the samples will not be as narrow as stated above; i.e., a few samples may be too low or too high for the range that was chosen for the majority of the samples. If so, the sample volume per tube may be adjusted up or down or the full scale deflection may be adjusted by means of the STD CAL.

Whenever possible, a cartridge configuration should be chosen such that adjustment of the STD CAL control will accommodate all the values encountered. Note that the STD CAL setting should not be changed while samples are being assayed; i.e., sample peaks can be compared to standard peaks only when both are run at the same STD CAL setting. If a STD CAL adjustment is anticipated, be sure to have on hand standards which will fall into the anticipated range.

### Standards

The recommended standards for use with the BD-40 are aqueous solutions of ammonium sulfate  $[(\text{NH}_4)_2\text{SO}_4]$  for nitrogen and potassium dihydrogen phosphate  $(\text{KH}_2\text{PO}_4)$  for phosphorus. The volumes of standard solution to be used depend on the concentration range of the sample.

It is recommended that two standards for each parameter to be run: one at 30 - 40% of full scale and one at 70 - 80% of full scale.

Standards should be handled in exactly the same manner as samples; i.e., they should be pipetted into the BD tubes and carried through the entire digestion procedure.

A series of working standard solutions which can accommodate all the ranges of the method can be prepared utilizing the following stock solutions:

#### Stock Solution A (2.0 mg N/ml)

Ammonium Sulfate  $(\text{NH}_4)_2\text{SO}_4$   
Distilled Water, q.s.

0.9434 g  
100 ml

### Preparation

Dissolve 0.9434 g of ammonium sulfate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.



**Stock Solution B** (2.0 mg P/ml)

Potassium Dihydrogen Phosphate ( $\text{KH}_2\text{PO}_4$ )	0.8788 g
Distilled Water, q.s.	100 ml

**Preparation**

Dissolve 0.8788 g of potassium dihydrogen phosphate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

The preparation of standards can be performed most readily if pipets ranging from 1 to 10 ml are available.

In Table #1, the extreme left column indicates the milliliters of stock solution to be diluted to one liter to obtain working standard solutions which will yield the concentration values in the second column. The same volumes diluted to 100 ml, or 10x those volumes diluted to one liter, will yield concentrations 10x these concentration values.

The preparation of standard solutions is best illustrated by an example. Using the example cited previously in the section on Samples, the N range was 1 - 5 mg/l and the P range was 5 - 50 mg/l.

For the N channel, an 80% of full scale standard would be 4.0 mg/l. From Table #1 4.0 mg/l can be obtained by using 8 ml per tube of a working standard solution containing 10 mg/l (Row 5, Column 8). Using 3 ml per tube of the same working solution will give 1.5 mg/l (Row 5, Column 3) or 30% of full scale.

Since 8 ml and 3 ml per tube were chosen for the nitrogen standards, the appropriate amount of P must also be present in those aliquots to accommodate the phosphorus channel. An 80% deflection for phosphorus corresponds to 40 mg P/l and a 30% deflection corresponds to 15 mg/l. Checking Column 8, 40 mg/l (10x chart value) can be obtained by using 8 ml stock solution B. The 30% value will automatically fall in range using the 3 ml aliquot. Hence, using 3 ml and 8 ml of a working standard solution prepared by diluting 5 ml of Stock Solution A plus 50 ml of Stock Solution B to one liter will yield the required N and P values.



The following general procedure may be used for preparation of standard solutions. Once the manifold configuration and concentration range have been chosen, choose a value (or 10x a chart value) from Table 1 which corresponds to an 80% deflection and which requires 5 ml per tube or more working standard solution. Using 5 ml or more for the 80% deflection insures that a smaller volume can be found on the chart which approximates the 30% deflection.

On a simultaneous system, either parameter may be determined first. Once the chart value has been chosen for one parameter, choose the value (or 10x a chart value) from the same column that most closely approximates an 80% deflection for the other parameter. Since the values are proportional to volume, the 30% values will automatically fall into range with each other.

### **Blanks**

A duplicate blank determination (all reagents less sample) should be performed with each rack of samples by carrying the blank tubes through the entire digestion procedure.

### **Operating Procedure**

#### **Samples, Standards and Blanks**

Samples and standards are pipetted directly into the digestion tubes. Samples should be pipetted in 20 ml aliquots. The amount of standard is determined by the level of the component(s) of interest.

Refer to Section II for guidelines on standard volumes and manifold configuration. Samples may be assayed singly or in duplicate, depending on workload. It is recommended that standards and blanks be assayed in duplicate.

While samples and standards are being prepared, pre-heat the block to 200°C by setting the HIGH TEMP dial to 200°C and depressing the MANUAL button.

### **Boiling Aids**

Plain (not selenized) Hengar chips are utilized to promote smooth boiling during digestion. The addition of 2 - 3 chips per tube is recommended. The use of glass beads or perforated glass beads is not satisfactory to obtain smooth boiling.

Hengar chips are available from Arthur H. Thomas Company, Vine & Third Streets,



Philadelphia, PA. 19105. As an alternative, some users report a preference for acid-washed Chemware TFE (teflon) boiling stones. TFE boiling stones are available from Markson Science, Inc., Box 767, Delmar, California 92014.

### Catalyst

Red mercuric oxide is recommended as a catalyst for the digestion of water samples. Because mercury can interfere in both chemistries, the amount added per tube is limited to an amount determined by the manifold configuration being utilized; i.e., the more the sample is diluted, the greater the amount of mercury that can be utilized. The amount of mercury recommended is 10 mg/tube. The mercury is most conveniently utilized as a solution of HgO in 10% sulfuric acid.

### Preparation

Into a 100 ml volumetric flask, weigh 8.0 of red mercuric oxide. Add about 75 ml of 10% sulfuric acid and stir until dissolved. Dilute to 100 ml with 10% sulfuric acid and mix thoroughly.

### Digestion (Salt/Acid/Catalyst) Mixture

To insure uniform blank values from tube to tube, it is recommended that potassium sulfate, sulfuric acid and catalyst be added to each tube as a single mixture rather than as separate components. The procedure below may be used to prepare the digestion mixture.

Prepare and store the mixture in a stoppered container to minimize the possibility of airborne contamination. The mixture may be prepared in as large a quantity as is practical to handle and store.

### Preparation

Carefully add 200 ml of concentrated sulfuric acid to 700 ml of de-ionized distilled water. Dissolve 133 g of potassium sulfate into this mixture, add 25 ml of mercuric sulfate catalyst solution and dilute to one liter with de-ionized distilled water.

For most applications, 5 ml of digestion mix per tube is satisfactory.



The utilization of a plunger-type repetitive dispensing device offers a rapid, convenient method of adding the digestion mixture to the tubes. When a plunger-type dispenser is utilized, the dispenser must be broken down, cleaned with water and air dried every three days. Failure to clean the plunger every three days can cause the plunger to freeze in the barrel of the dispenser due to crystallization of potassium sulfate.

### Digestion

After samples, standards and reagents have been added to the digestion tubes, the water must be evaporated before high temperature digestion can be performed.

Place the loaded rack into the pre-heated block (200°C) and attach the end plates to the rack. The plates should remain in place until the rack is removed from the block. End plates promote water evaporation during low temperature operation and insure proper refluxing of the acid during high temperature digestion.

When the loaded rack is placed in the block, set the programmer as follows and then depress the AUTO button:

Total Cycle Time:	2 1/2 hours
High Temp °C:	380 °C
Low Temp Time:	1 hour
Low Temp °C:	200 °C

Under these conditions, the unit will operate for a total cycle of 2 1/2 hours: 1 hour at 200 °C, about 1 hour to heat up to 380 °C and about 1/2 hour at 380 °C. At the end of 2 1/2 hours, the unit will automatically shut down.

### Cooling and Dilution

At the end of the program cycle (2 1/2 hours), remove the rack from the block, place it on an asbestos pad or in the cooling rack and remove the metal end plates. Allow the tubes to cool for about 5 minutes before diluting with 20 ml of de-ionized distilled water. Tubes are cool enough to dilute when the white acid fumes have dissipated and the upper half of the tube is cool enough to handle comfortably. The tubes should not be allowed to cool to the point of  $K_2SO_4$  precipitation.



With the aid of a vortex type tube mixer, add to each tube, while swirling, 20 ml of de-ionized distilled water using a repetitive pipetter. Add the water in one continuous portion at a moderate rate and angle the tube away from the face. Allow the tube contents to mix thoroughly.

The tube contents should be at room temperature before analyzing. The tubes may be cooled rapidly by placing the entire rack into a sink partially filled with cold water.

### **Analysis**

After cooling to room temperature, the digests may be analyzed using Bran + Luebbe Methodology No. 329-74W -- Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests, Revised 11/78.

Transfer to glass sample cups, which have been previously acid washed and dried.

Before analyzing the entire set of samples, standards and blanks, run a few standard cups through the system and with the STD CAL control, adjust the standard peaks to the proper chart reading.



**TABLE 1: CONCENTRATION OF STANDARD IN DIGESTS**

*MI Stock Solution A or B	Working Standard Conc. Mg N or P/L	MI Working Standard Solution Per Tube and Resulting Digest Concentration in Mg N or P/L									
		1	2	3	4	5	6	7	8	9	10
1	2	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
2	4	.2	.4	.6	.8	1.	1.2	1.4	1.6	1.8	2
3	6	.3	.6	.9	1.2	1.5	1.8	2.1	2.4	2.7	3
4	8	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4
5	10	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5
6	12	.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6
7	14	.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7
8	16	.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8
9	18	.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9
10	20	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10

\*Dilute to 1000 ml to get chart values

Dilute to 100 ml to get 10X chart values

Stock Solution A = Nitrogen

Stock Solution B = Phosphorus



**Appendix B-6 – Lab Procedures for Orthophosphate (PO<sub>4</sub> phosphate)**



**Table of Contents – Appendix B-6**

<b>Documents</b>
<b>QC Document – Orthophosphate</b>
<b>LACHAT Instruments – Quik Chem Method 10-115-01-1-A</b>
<b>LACHAT Instruments – Quik Chem Method 10-115-01-1-O</b>



## Orthophosphate

### 1.0 Procedure

Perform analysis for orthophosphate in accordance with procedures for the Lachat Quick Chem 8000 flow injection analyzer as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



# LACHAT

INSTRUMENTS

QuikChem Method 10-115-01-1-A

## Orthophosphate in Waters

0.01 to 2.00 mg P/L

### -- Principle --

The orthophosphate ion ( $\text{PO}_4^{3-}$ ) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

### -- Interferences --

1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg  $\text{SiO}_2/\text{L}$  would be required to produce a 0.005 mg P/L positive error in orthophosphate.
2. Concentrations of ferric iron ( $\text{Fe}^{3+}$ ) greater than 50 mg/L will cause a negative error due to precipitation of, and subsequent loss, of orthophosphate. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.
3. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

### -- Special Apparatus --

1. Heating Unit



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## QUIKCHEM METHOD 10-115-01-1-A

# DETERMINATION OF ORTHOPHOSPHATE BY FLOW INJECTION ANALYSIS COLORIMETRY

### 1. SCOPE AND APPLICATION

- 1.1. This method covers the determination of orthophosphate in drinking, ground, and surface waters, and domestic and industrial wastes. This method determines total orthophosphate or if the sample is filtered through a 0.45 micron pore size filter, the result is termed dissolved orthophosphate. The difference between the result of a sample determined directly and filtered is termed insoluble orthophosphate.
- 1.2. The method is based on reactions that are specific for the orthophosphate ( $\text{PO}_4^{3-}$ ) ion.
- 1.3. The applicable range is 0.01 to 2.00 mg P/L. The method detection limit is 0.01 mg P/L. Approximately 90 samples per hour can be analyzed.

### 2. SUMMARY OF METHOD

- 2.1. Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds are not recovered. The sulfuric acid in the molybdate reagent does not have enough contact time with polyphosphates to hydrolyze them.
- 2.2. The  $\text{PO}_4^{3-}$  reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of  $\text{PO}_4^{3-}$  in the digested sample.

### 3. DEFINITIONS

- 3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.
- 3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.



- 3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.
- 3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.10. PRACTICAL QUANTITION LIMIT (PQL) -- The lower level where measurements become quantitatively useful is called the PQL. The PQL is defined as  $PQL = 10 \cdot s$ , where  $s$  = the standard deviation of 21 replicates of a standard 2.5 - 5 times the MDL.
- 3.11. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.
- 3.12. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

#### **4. INTERFERENCES**

- 4.1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO<sub>2</sub>/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.
- 4.2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.



- 4.3. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 4.8 to 4.4% (v/v) H<sub>2</sub>SO<sub>4</sub> in the diluted digestion sample with no change in signal intensity.
- 4.5. For dissolved orthophosphate, sample turbidity must be removed by filtration prior to analysis. Sample color that absorbs at 880 nm will also interfere. When in doubt about background absorbance, the background concentration should be determined. See the System Notes section for more information.

## **5. SAFETY**

- 5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.
- 5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS.

5.3.1. Sulfuric acid

## **6. EQUIPMENT AND SUPPLIES**

- 6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.
- 6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.
- 6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.
  - 6.3.1. Autosampler
  - 6.3.2. Multichannel proportioning pump
  - 6.3.3. Reaction unit or manifold
  - 6.3.4. Colorimetric detector
  - 6.3.5. Data system
  - 6.3.6. Acid-washed glassware: All glassware used in the determination of phosphate should be washed with hot 1:1 HCl and rinsed with distilled water. Preferable, this



glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl is only required occasionally. Commercial detergent should never be used.

## **7. REAGENTS AND STANDARDS**

### **7.1. PREPARATION OF REAGENTS**

Use deionized water (10 megohm) for all solutions.

#### **Degassing with helium:**

To prevent bubble formation, degas the carrier solution with helium. Use He at 140 kPa (20 lb/in<sup>2</sup>) through a helium degassing tube (Lachat Part 50100). Bubble He vigorously through the solution for one minute.

#### **Reagent 1. Stock Ammonium Molybdate Solution**

**By Volume:** In a 1 L volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate**  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$  in approximately **800 mL water**. Dilute to the mark and invert three times. Store in plastic and refrigerate.

**By Weight:** To a tared 1 L container add **40.0 g ammonium molybdate tetrahydrate**  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$  and **983 g water**. Stir or shake until dissolved. Store in plastic and refrigerate.

#### **Reagent 2. Stock Antimony Potassium Tartrate Solution**

**By Volume:** In a 1 L volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot 1/2\text{H}_2\text{O}$ ) in approximately **800 mL of water**. Dilute to the mark and invert three times. Store in a dark bottle and refrigerate.

**By Weight:** To a 1 L dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot 1/2\text{H}_2\text{O}$ ) and **995 g water**. Stir or shake until dissolved. Refrigerate.



### Reagent 3. Molybdate Color Reagent

**By Volume:** To a 1 L volumetric flask add about **500 mL water**, then add **35.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 mL Stock Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark and invert three times. Degas with helium.

**By Weight:** To a tared 1 L container add **680 g water**, then **64.4 g concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 g Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 g Stock Ammonium Molybdate Solution** (Reagent 1). Shake and degas with helium.

### Reagent 4. Ascorbic Acid Reducing Solution, 0.33 M

**By Volume:** In a 1 L volumetric flask dissolve **60.0 g granular ascorbic acid** in about 700 mL of water. Dilute to the mark and invert to mix. Add **1.0 g dodecyl sulfate** ( $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$ ). Prepare fresh weekly. Discard if the solution becomes yellow.

**By Weight:** To a tared 1 L container, add **60.0 g granular ascorbic acid** and **975 g water**. Stir or shake until dissolved. Add **1.0 g dodecyl sulfate** ( $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$ ). Prepare fresh weekly. Discard if the solution becomes yellow.

### Reagent 5. Sodium Hydroxide - EDTA Rinse

Dissolve **65 g sodium hydroxide** ( $\text{NaOH}$ ) and **6 g tetrasodium ethylenediamine tetraacetic acid** ( $\text{Na}_4\text{EDTA}$ ) in 1.0 L or 1.0 kg water.

## 7.2. PREPARATION OF STANDARDS

### Standards for Simulated Digestion:

#### Standard 1. Stock Standard 250.0 mg P/L

In a 1 L volumetric flask dissolve **1.099 g primary standard grade anhydrous potassium phosphate monobasic** ( $\text{KH}_2\text{PO}_4$ ) that has been dried for one hour at  $105^\circ\text{C}$  in about **800 mL water**. Dilute to the mark with **DI water** and invert to mix.

#### Standard 2. Working Stock Standard Solution 5.00 mg P/L

**By Volume:** In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **DI water**. Invert to mix.

**By Weight:** To a tared **250 mL** container add about **5 g Stock Standard** (Standard 1). Divide the actual weight of the solution added by **0.02** and make up to this resulting total weight with **DI water**. Shake to mix.



### Set of Six Working Standards:

2.00	0.50	0.20	0.05	0.01	0.00	mg P/L
------	------	------	------	------	------	--------

**By Volume:** To six **250 mL** volumetric flasks add, respectively,

100.0	25.0	10.0	2.5	0.50	0.00	mL
-------	------	------	-----	------	------	----

**Standard 2.** Dilute each to the mark with DI water and invert to mix.

**By Weight:** To six tared **250 mL** containers add, respectively, about

100	25	10	2.5	0.50	0.0	g
-----	----	----	-----	------	-----	---

**Standard 2.** Take the exact weight obtained for each and divide by

0.4	0.1	0.04	0.01	0.002	0.0
-----	-----	------	------	-------	-----

to get the total weight of the diluted solution. Make up each solution to this total weight with DI water. Shake before using.

If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

## **8. SAMPLE COLLECTION, PRESERVATION AND STORAGE**

- 8.1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and acid rinsed. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.
- 8.2. The USEPA recommends that samples be filtered immediately upon collection, with a maximum holding time of 48 hours.

## **9. QUALITY CONTROL (USEPA GUIDELINE)**

- 9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

### **9.2. INITIAL DEMONSTRATION OF PERFORMANCE**

- 9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.



- 9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.
- 9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.
- 9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t = 2.528 for twenty one replicates]. S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

### 9.3. ASSESSING LABORATORY PERFORMANCE

- 9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.
- 9.3.2. Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.



- 9.3.3. The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery ( $\bar{X}$ ) and the standard deviation ( $S$ ) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \bar{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation ( $S$ ) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

- 9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within  $\pm 10\%$  of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within  $\pm 10\%$ . If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

#### 9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

- 9.4.1. Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.
- 9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$



Where,  $R$  = percent recovery,  $C_s$  = fortified sample concentration,  $C$  = sample background concentration,  $s$  = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

## **10. CALIBRATION AND STANDARDIZATION**

10.1. Prepare a series of at least 3 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution. (See section 7.2.)

10.2. Set up the manifold as shown in Section 17. Calibrate the instrument as described in section 11.

10.3. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.4. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed  $\pm 10\%$  of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

## **11. PROCEDURE**

### **11.1. PH ADJUSTMENT OF SAMPLES**

11.1.1. If samples of high ( $\text{pH} > 8$ ) are suspected add 1 drop of phenolphthalein indicator to a 50 mL aliquot of sample. If a red color develops, add 11 N sulfuric acid (310 mL concentrated  $\text{H}_2\text{SO}_4/\text{L}$ ) drop-wise to just discharge the color. Acid samples ( $\text{pH} < 4$ ) must be neutralized with 1 N NaOH (40 g NaOH/L).

### **11.2. CALIBRATION PROCEDURE (DATA SYSTEM PARAMETER)**

11.1.1. Prepare reagent and standards as described in section 7.

11.1.2. Set up manifold as shown in section 17.

11.1.3. Input data system parameters as in section 17.



- 11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 11.2.5. Place samples and/or standards in the autosampler. Input the information required by data system, such as concentration, replicates and QC scheme.
- 11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

### 11.3. SYSTEM NOTES

- 11.3.1. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free detergents for lab glassware.
- 11.3.2. Allow 15 min for heating unit to warm up to 37°C.
- 11.3.3. If necessary, at end of run place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (Reagent 10). Pump this solution for approximately 5 minutes to remove any precipitated reaction products. Then place these lines in water and pump for an additional 5 minutes. Then pump dry all lines.

## 12. DATA ANALYSIS AND CALCULATIONS

- 12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve.
- 12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3. Report results in mg P/L.

## 13. METHOD PERFORMANCE

- 13.1. The method performance data are presented as method support data in section 17. This data was generated according to Lachat Standard Operating Procedure J002, Lachat QC8000 FIA Support Data Generation or. Lachat Standard Operating Procedure J001, Lachat AE FIA Support Data Generation

## 14. POLLUTION PREVENTION

- 14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the



management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

- 14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 115 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

## **15. WASTE MANAGEMENT**

- 15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

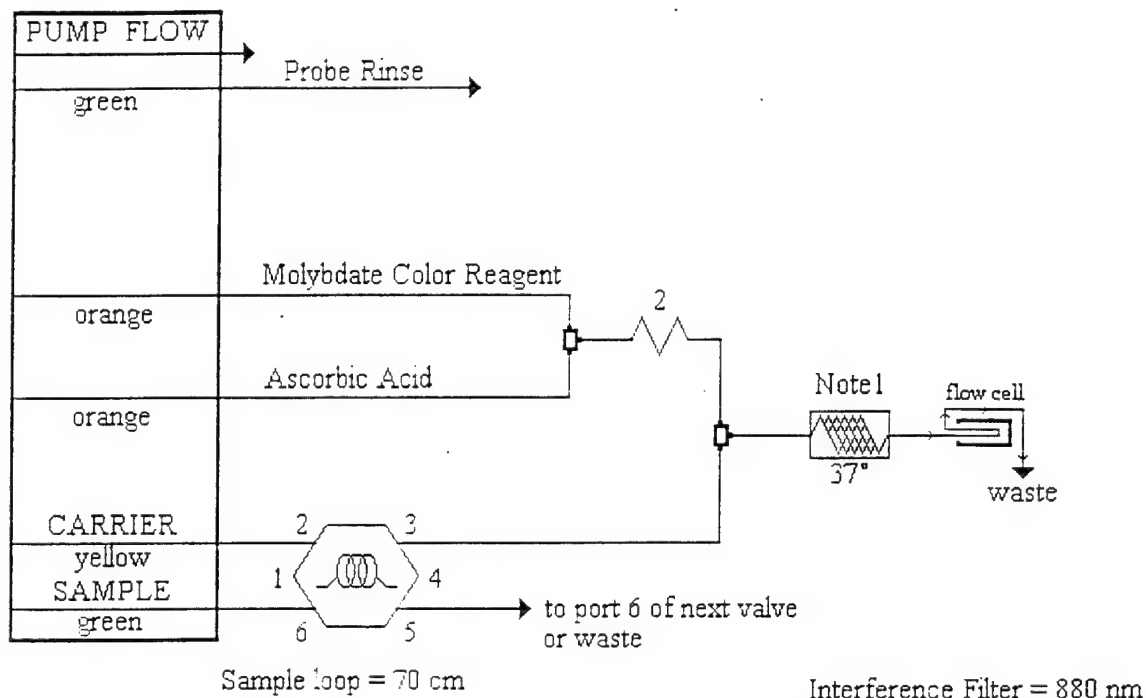
## **16. REFERENCES**

- 16.1. U.S. Environmental Protection Agency, Methods for the Determination of Inorganic Substances in Environmental Samples, EPA-600/R-93/100, August 1993, Method 365.1
- 16.2. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.
- 16.3. Standard Methods for the Examination of Water and Wastewater, 18th Edition, p. 4 - 116, Method 4500-P F (1992)
- 16.4. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.



## 17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA


### 17.1. ORTHOPHOSPHATE MANIFOLD DIAGRAM



**CARRIER** is DI water.

All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

2 is 135 cm of tubing on a 2 inch coil support

**APPARATUS:** An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at 37 °C.

**Note 1:** 175 cm of tubing on the heater.

**MANIFOLD DIAGRAM REVISION DATE:** 26 May 1992



## 17.2. DATA SYSTEM PARAMETERS FOR THE QUIKCHEM AE

### AE Parameters:

Sample Throughput: 90 samples/h, 40 s/sample

Pump Speed: 35 s

Cycle Period: 40 s

Inject to Start of Peak Period: 10 s

Top scale Response: 0.70 abs

Bottom Scale Response: 0.0 abs

Segment/Boundaries: A- 2.00 mg P/L

D- 0.05 mg P/L

F- 0.0 mg P/L

In the default RDF, change:

_Set	Default	Chord	0	to
_Set	Default	Chord	3	

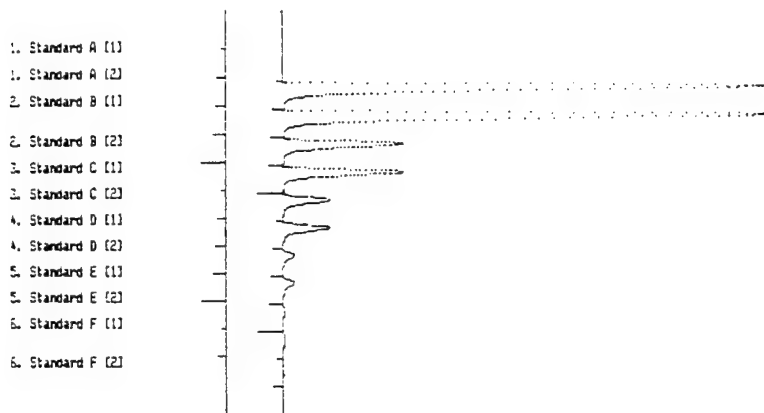
to sample and calibration RDFs.



## 17.3. SUPPORT DATA FOR QUIKCHEM AE

### 17.3.1. Calibration Peaks

Calibration 05/26/1992, 01:11 pm



### 17.3.2. MDL Determination

Rack 1 (Ref: 92052501) 05/26/1992, 01:29 pm

Dupe Sample ID	0-phosphate A mg P/L	MDL
101. 0.010 mg P/L (1)	0.009	x = 0.0088 mg P/L
102. 0.010 mg P/L (1)	0.009	s = 0.0007
103. 0.010 mg P/L (1)	0.010	MDL = 0.0018 mg P/L
104. 0.010 mg P/L (1)	0.009	
105. 0.010 mg P/L (1)	0.009	
106. 0.010 mg P/L (1)	0.010	
107. 0.010 mg P/L (1)	0.010	
108. 0.010 mg P/L (1)	0.009	
109. 0.010 mg P/L (1)	0.009	
110. 0.010 mg P/L (1)	0.009	
111. 0.010 mg P/L (1)	0.008	
112. 0.010 mg P/L (1)	0.008	
113. 0.010 mg P/L (1)	0.008	
114. 0.010 mg P/L (1)	0.009	
115. 0.010 mg P/L (1)	0.008	
116. 0.010 mg P/L (1)	0.008	
117. 0.010 mg P/L (1)	0.009	
118. 0.010 mg P/L (1)	0.008	
119. 0.010 mg P/L (1)	0.008	
120. 0.010 mg P/L (1)	0.008	
121. 0.010 mg P/L (1)	0.008	



### 17.3.3. Calibration Data

=====

QuikChem 42 Calibration Report for Calibration 92052601

Method: O-phosphate

This Calibration was done on 05/26/92 01:22 pm

This report prepared on 05/26/92 01:23 pm

=====

Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard A, O-phosphate A	ug P/L	2.000	1.999	0.05	0.3913
Standard B, O-phosphate A	ug P/L	0.500	0.504	-0.90	0.0992
Standard C, O-phosphate A	ug P/L	0.200	0.199	0.36	0.0396
Standard D, O-phosphate A	ug P/L	0.050	0.050	-0.15	0.0099
Standard E, O-phosphate A	ug P/L	0.010	0.010	2.63	0.0020
Standard F, O-phosphate A	ug P/L	0.000	0.000	***.00	0.0001

----- end of Calibration Report for Calibration 92052601 -----

Cal Ref: 92052601  
Method: O-PHOS

Calibration Statistics Report  
05/26/92 01:23 pm

Channel: O-phosphate A

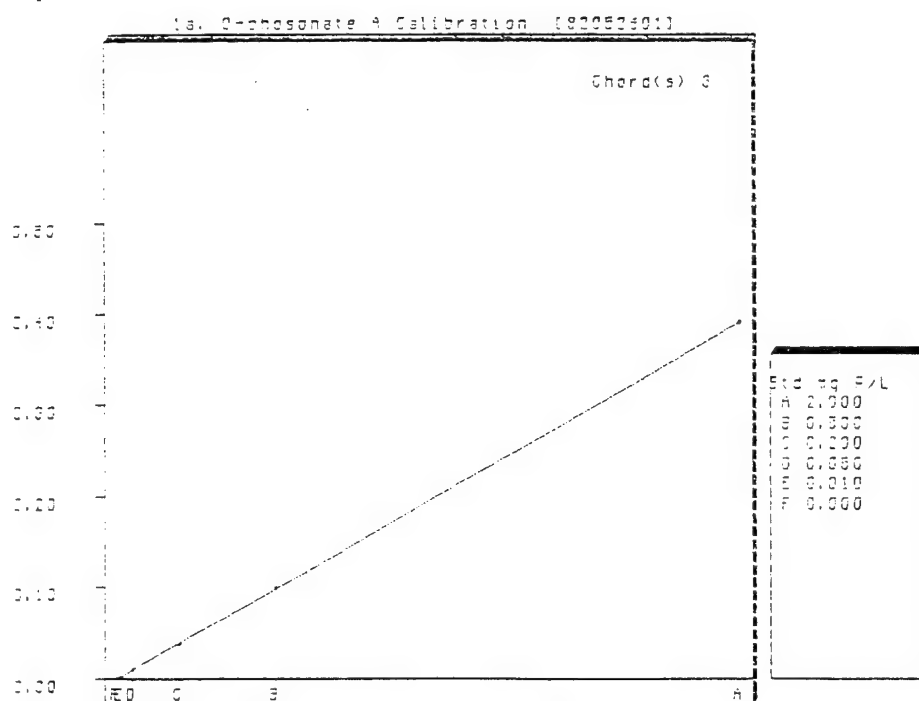
Correlation Coefficients

Seg Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1 A-D	1.0000	0.9999	1.0000	1.0000	0.9998	0.9629 ?
2 D-F	0.9980	0.9412 ?	0.9995	0.9996	0.9794 ?	0.0514 ?

Percent Standard Deviation in Slope

1 A-D	0.1	0.5	0.1	0.2	0.5	8.0
2 D-F	2.2	12.5	1.1	1.0	7.2	214.9 ?

### 17.3.4. Calibration Graph

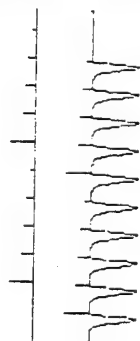




### 17.3.5. Precision Determination

Rack 1 (Ref: 92052602) 05/26/1992, 01:45 pm

101. 0.20 ug P/L (1)  
102. 0.20 ug P/L (1)  
103. 0.20 ug P/L (1)  
104. 0.20 ug P/L (1)  
105. 0.20 ug P/L (1)  
106. 0.20 ug P/L (1)  
107. 0.20 ug P/L (1)  
108. 0.20 ug P/L (1)  
109. 0.20 ug P/L (1)  
110. 0.20 ug P/L (1)



Cup# Sample ID

101 0.20 ug P/L  
102 0.20 ug P/L  
103 0.20 ug P/L  
104 0.20 ug P/L  
105 0.20 ug P/L  
106 0.20 ug P/L  
107 0.20 ug P/L  
108 0.20 ug P/L  
109 0.20 ug P/L  
110 0.20 ug P/L

O-phosphonate A  
ug P/L

0.197  
0.195  
0.195  
0.195  
0.195  
0.195  
0.198  
0.198  
0.198  
0.198

Precision

$\bar{x} = 0.197$  ug P/L

$s = 0.0011$

RSD = 0.58%

### 17.3.6. Carryover

Rack 1 (Ref: 92052603) 05/26/1992, 01:54 pm

101. 2.0 ug P/L (1)  
102. blank (1)  
103. blank (1)  
104. blank (1)  
105. blank (1)  
106. blank (1)  
107. blank (1)  
108. blank (1)  
109. blank (1)  
110. blank (1)  
111. blank (1)



Cup# Sample ID

101 2.0 ug P/L  
102 blank  
103 blank  
104 blank  
105 blank  
106 blank  
107 blank  
108 blank  
109 blank  
110 blank  
111 blank

O-phosphonate A  
ug P/L

2.003  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014  
0.0014

Carryover

$\bar{x} = 0.0014$

$s = 0.0007$

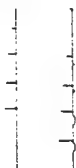
$t = 2.036$

95% CI = 0.0014 +/- 0.0005

### 17.3.7. Silicate Interference

Rack 1 (Ref: 92052605) 05/26/1992, 02:09 pm

101. 30.0 ug SiO2/L (1)  
102. 30.0 ug SiO2/L (1)  
103. 50.0 ug SiO2/L (1)  
104. 50.0 ug SiO2/L (1)



Cup# Sample ID

101 30.0 ug SiO2/L  
102 30.0 ug SiO2/L  
103 50.0 ug SiO2/L  
104 50.0 ug SiO2/L

1  
O-phosphonate A  
ug P/L

0.0054  
0.0064  
0.011  
0.009

Silica Interference: The ratio of SiO<sub>2</sub> to P is defined as the selectivity

Selectivity

6000  
5000  
4500  
5500



#### 17.4. DATA SYSTEM PARAMETERS FOR QC 8000

##### **Analyte Data:**

Chemistry: Direct

Peak Base Width: 25 s

% Width Tolerance: 100

Threshold: 10000

Inject to Peak Start: 10 s

##### **Calibration Data:**

Calibration Rep Handling: Weighted Avg

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

##### **Sampler Timing:**

Min. Probe in Wash Period: 9 s

Probe in Sample Period: 20 s

##### **Valve Timing:**

Load Period: 15 s

Inject Period: 25 s

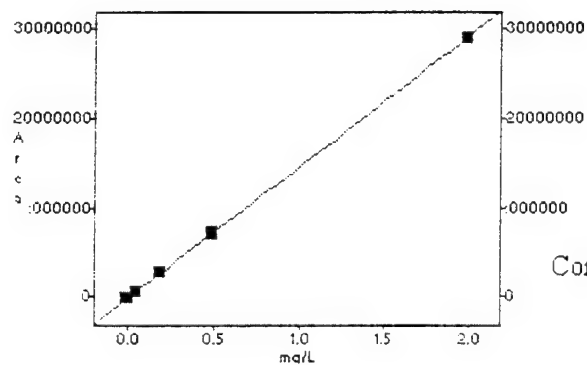


## 17.5. SUPPORT DATA FOR QC 8000

### 17.5.1. Calibration Report

Standard (mg P/L)	Determined	RSD %	Residual %	Area 1	Area2
2.00	2.00	<0.1	0.0	29246438	29240522
0.50	0.50	0.1	-0.7	7369013	7358164
0.20	0.21	0.1	-0.3	2931633	2937131
0.05	0.05	0.4	4.8	697248	693182
0.01	0.01	<0.1	9.3	131525	131593
0.00	0.00	--	--	0	0

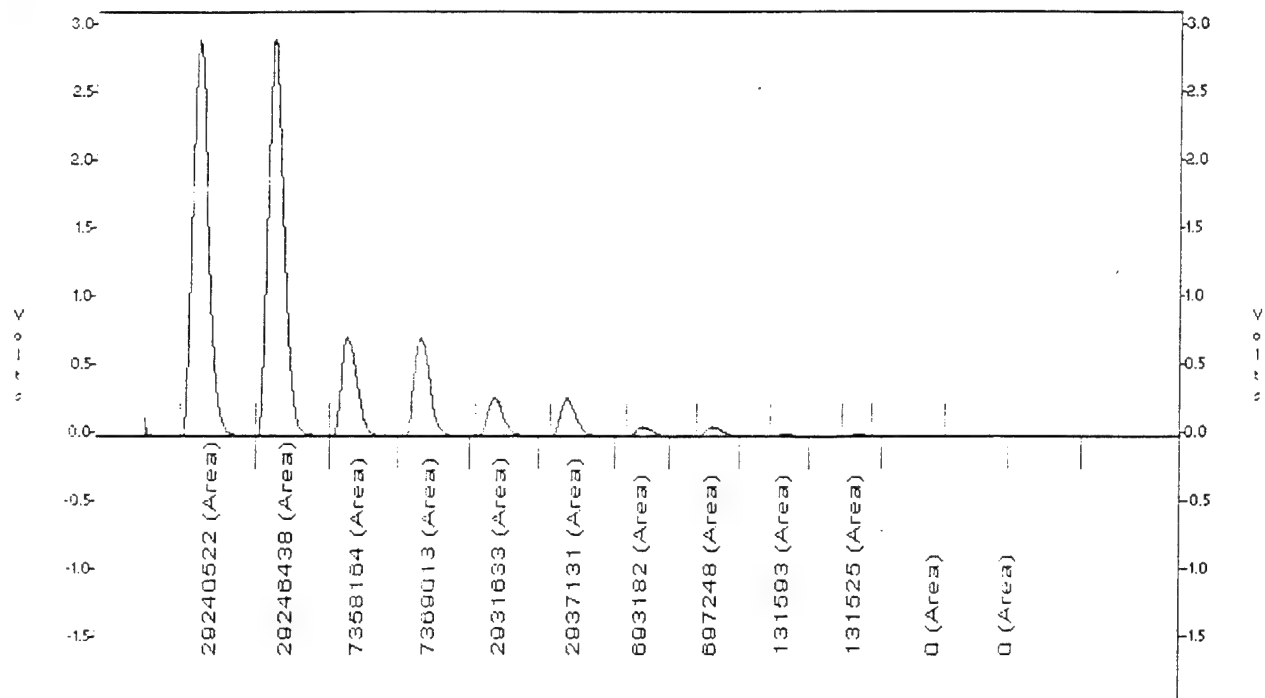
### 17.5.2. Calibration Graph



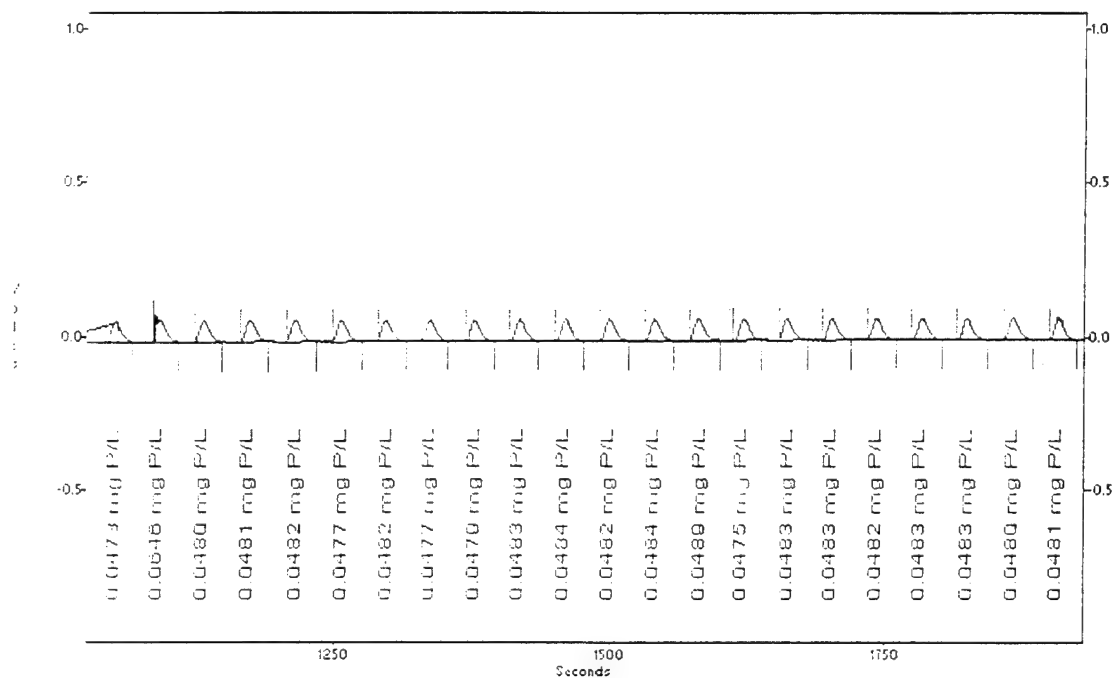
Scaling: none  
 Weighting: none  
 1st Order Poly  
 $\text{Conc} = 6.84\text{e-}8\text{Area} + 8.02\text{e-}5$   
 $R^2 = 1.0000$



### 17.5.3. Calibration Peaks



### 17.5.4. MDL Determination

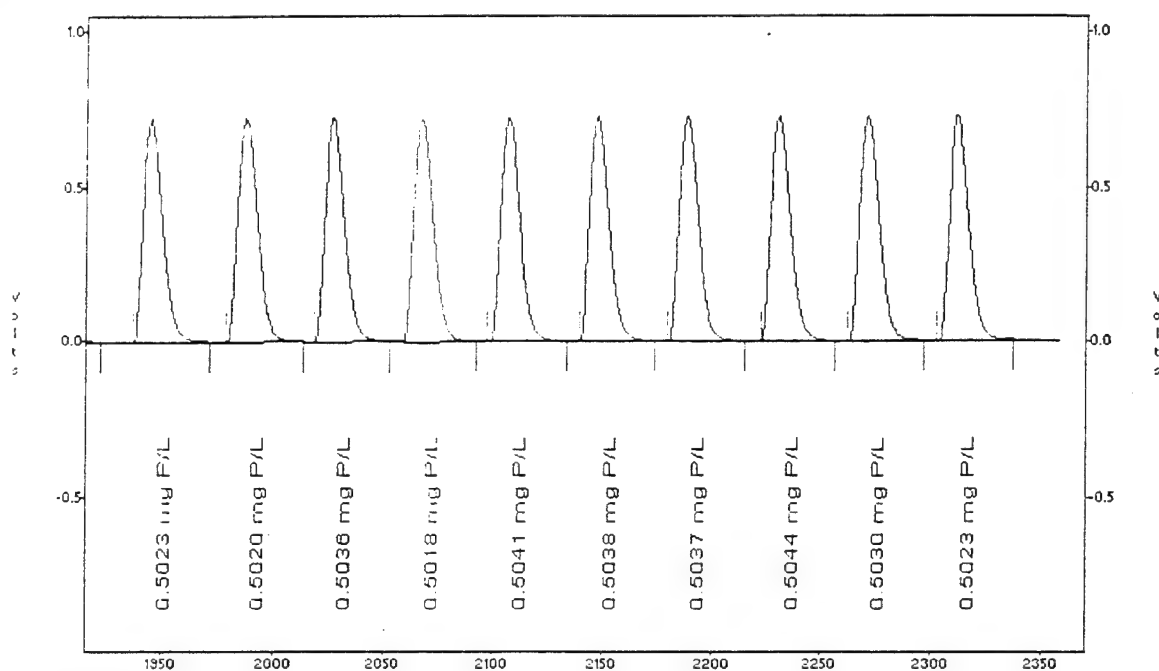


Replicates of 0.05 mg P/L

Mean = 0.049 mg P/L, Std. Dev.(s) = 0.004 mg P/L, MDL =  $2.52 \times s = 0.009$  mg P/L



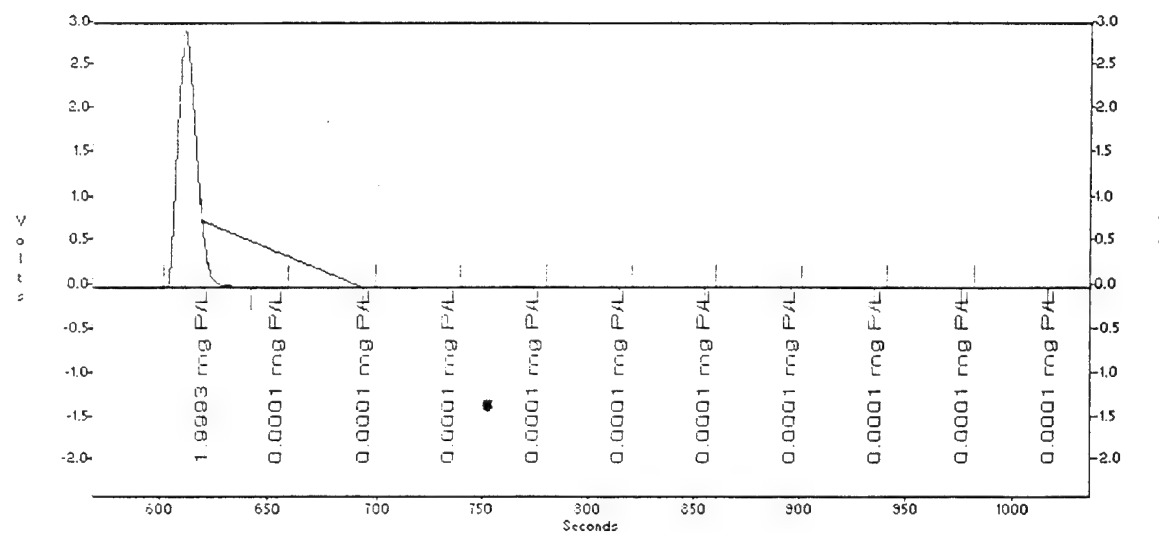
### 17.5.5. Precision Determination



10 Replicates of 0.50 mg P/L

Mean = 0.503 mg P/L, Std. Dev.(s) = 0.001 mg P/L, % RSD = 0.186

### 17.5.6 Carryover



All blanks were not detected (area = 0) at threshold = 10,000.



## QuikChem Method No. 10-115-01-1-O

**PARAMETER:** Orthophosphate

**MATRIX:** Potable and surface waters, domestic and industrial wastewater

**RANGE:** 0.10 to 20.0 mg P/L as  $\text{PO}_4^{3-}$

### **SAMPLE**

**THROUGHPUT:** 100 samples/h; 36 s/sample

### **PRINCIPLE:**

The orthophosphate ion ( $\text{PO}_4^{3-}$ ) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

### **INTERFERENCES:**

1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.
2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.
3. The determination of phosphorus is sensitive to variations in acid concentrations in the sample. The higher the acidity, the smaller the sensitivity of the method is. A careful balance of acid concentrations is required among samples, standards, and blanks.
4. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

### **SAMPLE HANDLING AND PRESERVATION:**

Samples should be stored at 4°C and determined as soon as possible after collection. If sulfuric acid preservation is used, hydrolysis of any polyphosphate species in the sample will occur. If the samples are to be stored for long periods, they can be frozen.

### **REAGENT PREPARATION RECIPES:**

Use deionized water (10 megohm) for all solutions.

1. Degassing with Helium



To prevent bubble formation, degas all solutions except the standards with helium. Use He at 20 lb/in<sup>2</sup> through a fritted gas dispersion tube. Bubble He vigorously through the solution for one minute.

## 2. Stock Ammonium Molybdate Solution

**By Volume:** In a 1 L volumetric flask dissolve 40.0 g of ammonium molybdate tetrahydrate  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$  in approximately 800 mL of water. Dilute to the mark and invert three times. Store in plastic and refrigerate.

**By Weight:** To a tared 1 L container add 40.0 g ammonium molybdate tetrahydrate  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$  and 983 g water. Stir or shake until dissolved. Store in plastic and refrigerate.

## 3. Stock Antimony Potassium Tartrate Solution

**By Volume:** In a 1 L volumetric flask, dissolve 3.0 g of antimony potassium tartrate [potassium antimonyl tartrate hemihydrate  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot 1/2\text{H}_2\text{O}]$  in approximately 800 mL of water. Dilute to the mark and invert three times. Store in a dark bottle and refrigerate.

**By Weight:** To a 1 L dark, tared container add 3.0 g antimony potassium tartrate [potassium antimonyl tartrate hemihydrate  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot 1/2\text{H}_2\text{O}]$  and 995 g water. Stir or shake until dissolved. Refrigerate.

## 4. Molybdate Color Reagent

**By Volume:** To a 1 L volumetric flask add about 500 mL water, then add 70.0 mL of concentrated sulfuric acid (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add 72.0 mL of the Stock Antimony Potassium Tartrate Solution (3., above) and 213 mL of the Stock Ammonium Molybdate Solution (2., above). Dilute to the mark and invert three times. Degas with helium.

**By Weight:** To a tared 1 L container add 644 g water, then 128.8 g concentrated sulfuric acid (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add 72.0 g of the Stock Antimony Potassium Tartrate Solution (3., above) and 213 g of the Stock Ammonium Molybdate Solution (2., above). Shake and degas with helium.

## 5. Ascorbic Acid Reducing Solution

**By Volume:** In a 1 L volumetric flask dissolve 60.0 g ascorbic acid in about 700 mL of water. Dilute to the mark and invert three times. Degas. Dissolve 1.0 g dodecyl sulfate, sodium salt  $(\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na})$ . Prepare fresh weekly.

**By Weight:** To a tared 1 L container, add 60.0 g ascorbic acid and 975 g water. Stir or shake until dissolved. Degas. Dissolve 1.0 g dodecyl sulfate, sodium salt  $(\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na})$ . Prepare fresh weekly.



## 6. Sodium Hydroxide - EDTA Rinse

Dissolve 65 g of sodium hydroxide (NaOH) and 6 g of tetrasodium ethylenediamine tetraacetic acid ( $\text{Na}_4\text{EDTA}$ ) in 1.0 L or 1.0 kg of water.

## STANDARDS PREPARATION RECIPES:

### 1. Stock Standard 200.0 mg P/L as $\text{PO}_4^{3-}$

In a 1 L volumetric flask dissolve 0.8788g of anhydrous potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) which has been dried at  $105^\circ\text{C}$  in approximately 800 mL of water. Dilute to the mark and invert three times.

### 2. Working Stock Standard 20.00 mg P/L as $\text{PO}_4^{3-}$

**By Volume:** Into a 1 L volumetric flask pipet 100.00 mL of the Stock Standard in 1. Dilute to the mark and invert three times.

**By Weight:** To a tared 1 L container add about 100 g of the Stock Standard Solution in 1. Measure the weight of this solution added and divide this weight by 0.1. This will give you the total weight of the dilution solution to be made. Make up the solution to this weight with water. Shake before using.

### 3. Working Standards, Set of Six: 0.20 to 10.0 mg P/L as $\text{PO}_4^{3-}$

A subset of these standards can be used depending on the application.

**By Volume:** To six 100 mL volumetric flasks add, respectively,

50.00, 25.00, 10.00, 5.00, 2.50, and 1.0 mL

of the Working Stock Standard (20.0 mg/l). Dilute each to the mark and invert three times. This makes standards of, respectively,

10.00, 5.00, 2.00, 1.00, 0.50, and 0.20 mg P/L as  $\text{PO}_4^{3-}$

**By Weight:** To six tared 100 mL containers add, respectively, about

50, 25, 10, 5, 2.5, and 1.0 g

of the Working Stock Standard (20.0 mg/l). For each in turn, measure the exact weight of solution added and divide this weight by

0.5, 0.25, 0.1, 0.05, 0.025, and 0.01



respectively. This will, in turn, give you the total weight of the diluted solution to be made. Make up each solution to this total weight with water, using a wash bottle filled with water for the last 10 g or so. This makes standards of, respectively,

10.00, 5.00, 2.00, 1.00, 0.50, and 0.20 mg P/L as  $\text{PO}_4^{3-}$

If samples often fall within a smaller range, more standards within this smaller range can be added and standards outside this smaller range can be dropped.

**APPARATUS:** Lachat QuikChem Automated Flow Injection Ion Analyzer which includes:

- A. Automatic Sampler
- B. Proportioning Pump
- C. Injection Module with a 11 cm 0.81 mm i.d. sample loop
- D. Colorimeter
  - 1. Flow Cell: 10 mm, 80 ul
  - 2. Interference Filter, 880 nm
- E. Heating Cell with temperature controller. 650 cm x 0.81 mm i.d. tubing.
- F. Reaction Module 10-115-01-1-O
- G. QuikCalc Software or chart recorder

**INJECTION TIMING:**

Pump speed: 35  
Cycle period: 36 s  
Load period: 18 s  
Inject period: 18 s  
Inject to start of peak period: 26 s  
Inject to end of peak period: 58 s

**GAIN:**

Gain = 090 x 10

**SYSTEM OPERATION:**

- A. Inspect all modules for proper connections.
- B. Turn on power and all modules. Allow heating cell to warm up to 60°C.



- C. Place reagent feedlines into proper containers. Raise tension levers on pump tube cassettes.
- D. Pump system until a stable baseline is attained.
- E. Program data system to initial parameters or those empirically determined.
- F. Place calibration standards and blank in sample tray in descending order of concentration followed by unknowns and check standards.
- G. At end of run place the carrier and reagent lines into the NaOH-EDTA solution (6. Reagents, above). Pump this solution for approximately 5 minutes to remove precipitated reaction products. Then place these lines in water and pump for an additional 5 minutes. Then pump dry all lines.
- H. Turn off pump, all modules, and release levers on pump tube cassettes.

**SOURCES:**

U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 365.3

**Methods for Determination of Inorganic Substances in Water and Fluvial Sediments.** Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

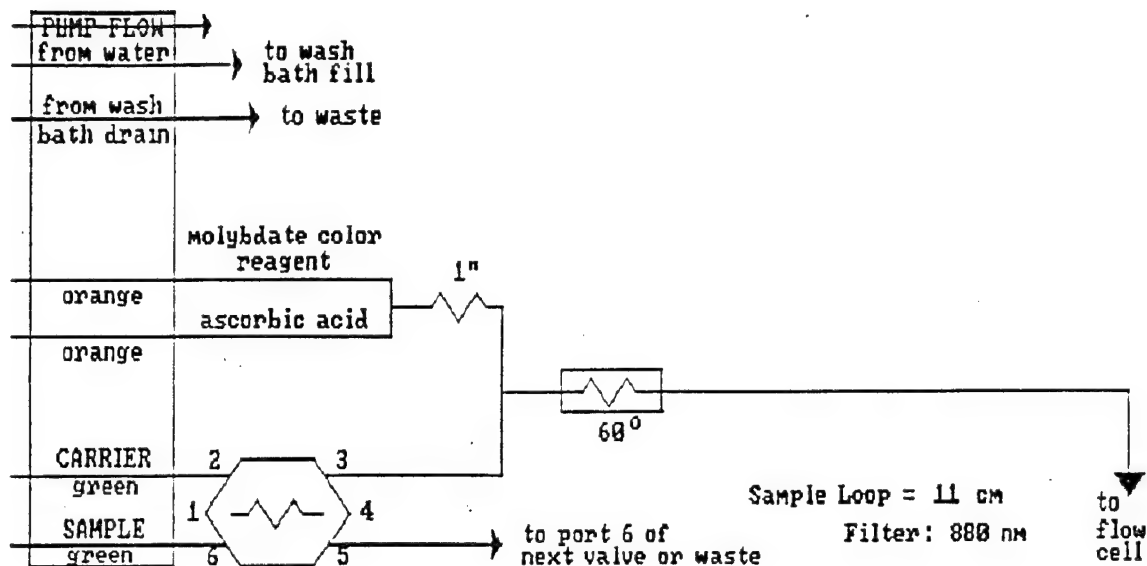
**REVISION:** 07 March 1990 A. Bloxham

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Manifold Diagram:



CARRIER is helium degassed water.

1"	is	70.0	cm of tubing on a 1 in coil support
2"	is	135	cm of tubing on a 2 in coil support
2.5"	is	168	cm of tubing on a 2.5 in coil support
3"	is	202	cm of tubing on a 3 in coil support
4"	is	255	cm of tubing on a 4 in coil support
8"	is	550	cm of tubing on a 8 in coil support

Heated tubing is shown inside a box with the temperature next to the box. Heated tubing is 650 cm unless otherwise specified.

All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

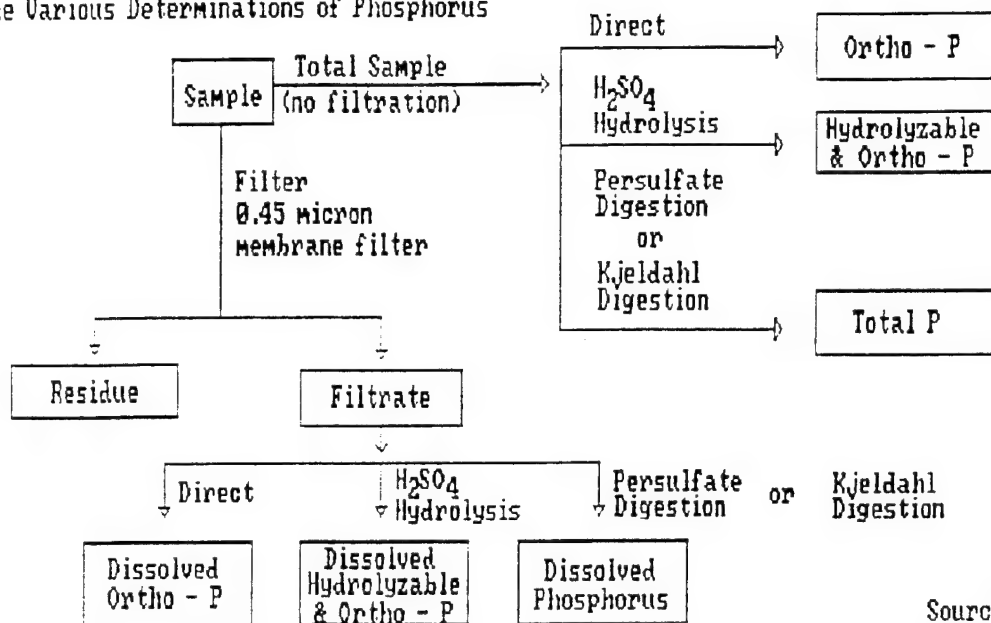
Notes:

MANIFOLD DIAGRAM REVISION DATE: 07 March 1990



SYSTEM NOTE:

The Various Determinations of Phosphorus



Source: US EPA



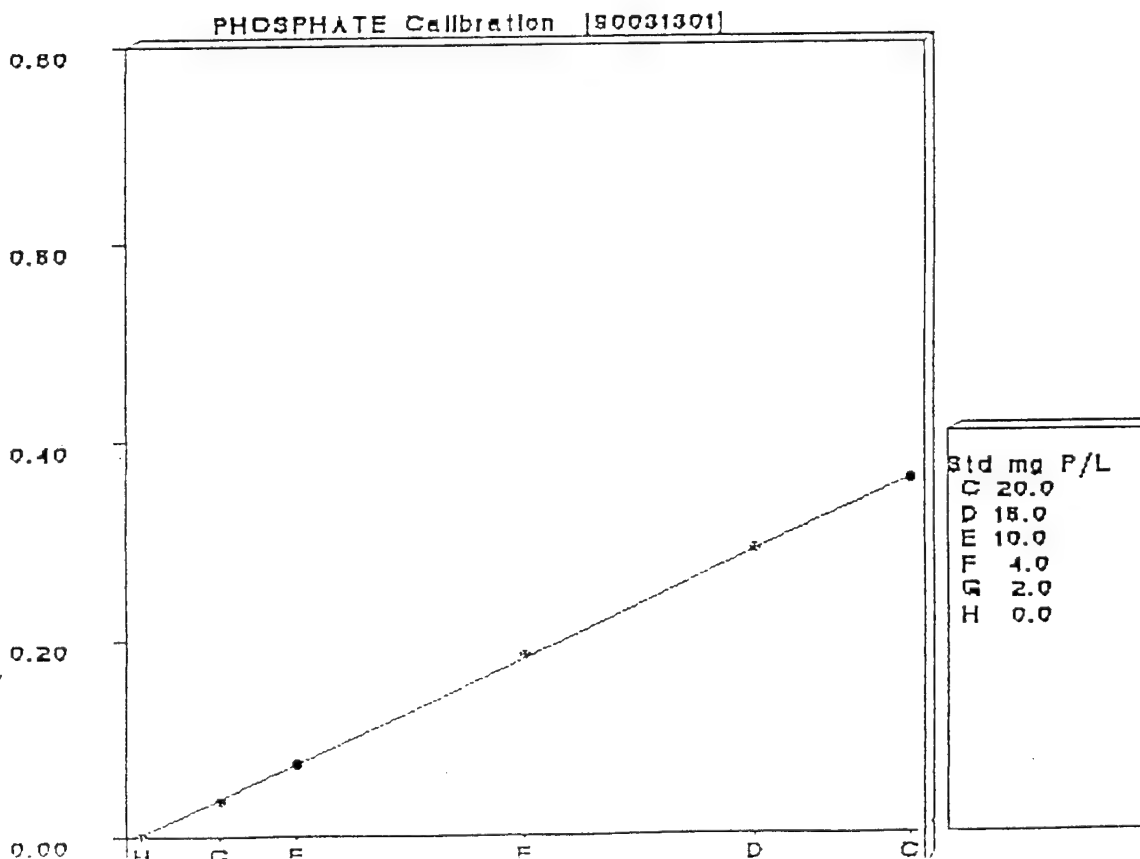
=====

QuikChem AE Calibration Report for Tray Reference 90031301.RC

This report was prepared on 03/31/90 at 03:09 pm  
This calibration was done on 03/13/90 at 05:28 pm

=====

Standard	Concentration	%	Residual	Absorbance	PIF
Standard C:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 20.000 19.918		-0.41	0.745	0.99
Standard D:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 18.000 15.978		-0.14	0.595	1.00
Standard E:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 10.000 10.191		1.91	0.376	0.99
Standard F:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 4.000 4.023		0.58	0.146	0.98
Standard G:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 2.000 1.991		-0.43	0.071	0.98
Standard H:	-----	-----			
Analyte	Units Known Determined				
PHOSPHATE	mg P/L 0.000 -0.102		****	****	0.00





**Appendix B-7 – Lab Procedures for Nitrate + Nitrite Nitrogen (NO<sub>3</sub>+NO<sub>2</sub> Nitrogen)**



## **Table of Contents – Appendix B-7**

<b>Documents</b>
<b>QC Document – Nitrate + Nitrite Nitrogen</b>
<b>LACHAT Instruments – Quik Chem Method 10-107-04-1-A</b>
<b>LACHAT Instruments – Quik Chem Method 10-107-04-1-C</b>
<b>LACHAT Instruments – Quik Chem Method 10-107-04-1-D</b>
<b>Technicon Auto Analyzer II – Industrial Method No. 100-70W/B</b>



## Nitrogen, Total Kjehldahl - Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

### 1.0 Procedure

Perform analysis for Total Kjehldahl Nitrogen (Method 351.2) in accordance with procedures for the Technicon II AutoAnalyzer, or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



QuikChem Method 10-107-04-1-A

## **Nitrate/Nitrite, Nitrite in Surface Water, Wastewater**

0.2 to 20.0 mg N/L as  $\text{NO}_3^-$  or  $\text{NO}_2^-$

### **-- Principle --**

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column.

### **-- Interferences --**

1. Residual chlorine can interfere by oxidizing the cadmium column.
2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.
3. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.
4. Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis.

### **-- Special Apparatus --**

1. Cadmium-Copper Reduction Column (Lachat Part # 50237)

Revised by Karin Wendt/wrp and Copyrighted (c) on 11 Aug 1994 lc by **Lachat Instruments, 6645 West Mill Road, Milwaukee, WI 53218, USA**. Phone: 1-414-358-4200 FAX: 1-414-358-4206. This document is the property of Lachat Instruments. Unauthorized copying of this document is prohibited.



### -- Sample Handling and Preservation --

Nitrite will be oxidized by air to nitrate in a few days. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc. H<sub>2</sub>SO<sub>4</sub> per liter) and refrigerated. **CAUTION:** Samples must not be preserved with mercuric chloride or thiosulfate because this will degrade the cadmium column.

If build-up of suspended matter in the reduction column restricts sample flow, the samples may be pre-filtered.

### -- Preparation of Reagents --

Use deionized water (10 megohm) for all solutions.

#### Reagent 1. 15 N Sodium hydroxide

In a 1 L container, add **150 g NaOH** very slowly to **250 mL of water**. **CAUTION:** The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

#### Reagent 2. Ammonium chloride buffer, pH 8.5

**By Volume:** In a 1 L volumetric flask, dissolve **85.0 g ammonium chloride (NH<sub>4</sub>Cl)** and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate (Na<sub>2</sub>EDTA·2H<sub>2</sub>O)** in about **800 mL water**. Dilute to the mark and invert to mix. Adjust the pH to 8.5 with **15 N sodium hydroxide**.

**By Weight:** To a tared 1 L container, add **85.0 g ammonium chloride (NH<sub>4</sub>Cl)**, **1.0 g disodium ethylenediamine tetraacetic acid dihydrate (Na<sub>2</sub>EDTA·2H<sub>2</sub>O)** and **938 g water**. Shake or stir until dissolved. Then adjust the pH to 8.5 with **15 N sodium hydroxide**.

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. An alternative recipe for the ammonium chloride buffer is:

**By Volume: CAUTION: Fumes!!!** In a hood, to a 1 L volumetric flask add **500 mL water**, **105 mL concentrated hydrochloric acid (HCl)**, **62 mL ammonium hydroxide (NH<sub>4</sub>OH)**, and **1.0 g disodium EDTA**. Dissolve and dilute to the mark. Invert to mix. Adjust the pH to 8.5 with **HCl** or **15 N NaOH**.



**By Weight:** CAUTION: Fumes!!! In a hood, to a tared 1 L container add **800 g water**, **126 g concentrated hydrochloric acid (HCl)**, **55.6 g ammonium hydroxide (NH<sub>4</sub>OH)** and **1.0 g disodium EDTA**. Stir until dissolved. Adjust the pH to 8.5 with HCl or 15 N NaOH.

### Reagent 3. Sulfanilamide color reagent

**By Volume:** To a volumetric flask add about 800 mL water. Then add 100 mL of 85% phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet, and stir to dissolve for 30 min. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

**By Weight:** To a tared, dark 1 L container add **876 g water**, **170 g 85% phosphoric acid (H<sub>3</sub>PO<sub>4</sub>)**, **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED)**. Shake until wetted and stir with stir bar for 30 min. until dissolved. This solution is stable for one month.

### -- Preparation of Standards --

**NOTE:** Following are standards preparations for a 1 channel system determining NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> or NO<sub>2</sub><sup>-</sup> and a 2 channel system where one channel is used for NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> and the other channel is used for determining NO<sub>2</sub><sup>-</sup>. For the 1 channel system, either NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup> standards may be used. We recommend the use of NO<sub>3</sub><sup>-</sup> standards when running a 1 channel method for NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>. For the 2 channel system, we recommend the use of both NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> standard sets.

#### Standard 1. Stock Nitrate Standard 200.0 mg N/L as NO<sub>3</sub><sup>-</sup>

In a 1 L volumetric flask dissolve **1.444 g potassium nitrate (KNO<sub>3</sub>)** in about **600 mL water**. Add **2 mL chloroform**. Dilute to the mark and invert to mix. This solution is stable for six months.

#### Standard 2. Stock Nitrite Standard 200.0 mg N/L as NO<sub>2</sub><sup>-</sup>

**By Volume:** In a 1 L volumetric flask dissolve **0.986 g sodium nitrite (NaNO<sub>2</sub>)** or **1.214 g potassium nitrite (KNO<sub>2</sub>)** in approximately **800 mL water**. Add **2 mL chloroform**. Dilute to the mark and invert to mix. Refrigerate.

#### Set of Six Working NO<sub>2</sub><sup>-</sup> Standards:

20.0   8.0   4.0   1.00   0.40   0.20   mg N/L



**By Volume:** To six **250 mL** volumetric flasks add, respectively,

**25.0 10.0 5.00 1.25 0.50 0.25 mL**

of the **Stock Standard 2**. Dilute each to the mark with **water** and invert to mix.

**By Weight:** To six **250 mL** containers add, respectively, about

**25 10 5 1.25 0.50 0.25 g**

of the **Working Stock Standard 2**. Take the exact weight obtained for each and divide by

**0.1 0.04 0.02 0.005 0.002 0.001**

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

**Set of Six Working NO<sub>3</sub><sup>-</sup> Standards:**

**20.0 8.0 4.0 1.00 0.40 0.20 mg N/L**

**By Volume:** To six **250 mL** volumetric flasks add, respectively,

**25.0 10.0 5.00 1.25 0.50 0.25 mL**

of the **Working Stock Standard 1**. Dilute each to the mark with **water** and invert to mix.

**By Weight:** To six **250 mL** containers add, respectively, about

**25 10 5 1.25 0.50 0.25 g**

of the **Working Stock Standard 1**. Take the exact weight obtained for each and divide by

**0.1 0.04 0.02 0.005 0.002 0.001**

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.



## -- Preparation of Reagents for Cadmium Reduction Column --

### Reagent A. 1 M Hydrochloric Acid (HCl)

**By Volume:** In a 100 mL container, add 8 mL concentrated HCl to 92 mL water. Stir or shake to mix.

**By Weight:** To a 100 mL container, add 92 g water then add 9.6 g concentrated HCl. Stir or shake to mix.

### Reagent B. 2% Copper Sulfate Solution

**By Volume:** In a 1 L volumetric flask, dissolve 20 g copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in about 800 mL water. Dilute to mark with water. Invert to mix thoroughly.

**By Weight:** To a 1 L container, add 20 g copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to 991 g water. Stir or shake to dissolve.

## -- Preparation of Cadmium Reduction Column --

### Cadmium Preparation

Place 10-20 g of coarse cadmium granules (0.3 - 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with 50 mL of acetone, then water, then two 50 mL portions of 1 N hydrochloric acid (Reagent A). Rinse several times with water.

**CAUTION:** Collect and store all waste cadmium. **Cadmium is toxic and carcinogenic.** Wear gloves and follow the precautions described on the Material Safety Data Sheet.

### Copperization

Add a 100 mL portion of 2% Copper Sulfate Solution (Reagent B) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh 100 mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (Reagent 2) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a stoppered bottle under ammonium chloride buffer (Reagent 2).

### Packing the Column

The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that your hands are free. Unscrew one of the colored fittings from an end of the column, and pull out and save the foam plug. The column



and threads are glass so be careful not to break or chip them. Fasten this fitting up higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer (Reagent 2).

Scoop up prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with DI water.

If air remains in the column or is introduced accidentally, connect the column into the manifold, turn the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

### **-- Cadmium Column Insertion Procedure --**

- a. Before inserting the column, pump all reagents into manifold.
- b. Turn the pump off and immediately connect to the outlet tubing of the buffer mixing coil.
- d. Connect the open tubing on the column to the tee fitting where the color reagent is
- e. Return the pump to normal speed.
- f. The direction of reagent flow through the column is not relevant.

### **Column Efficiency Procedure**

- a. Visually inspect the column. Check for air bubbles in the column or lines, gaps in the column or any change in the cadmium surface characteristics. (cadmium granules should be dark gray).
- b. If air bubbles are present in column, connect the column into the manifold, turn the pump on maximum and tap firmly with a screwdriver handle, being careful not to break the column, working up the column until all air is removed. If air cannot be removed, the column should be repacked. Cadmium columns should be stored filled with buffer. If air enters the column, efficiency will decrease.
- c. Check the flow efficiency by disconnecting the cadmium column from the manifold and reconnecting to a green pump tube. Pump buffer through the packed column and collect in a graduated cylinder. The flow rate with the column connected should be greater than 4.0 mL/min.
- d. There are two procedures for determining column efficiency, as follows:



**Slope Ratio Method**

1. Calibrate with the mid-range NO<sub>3</sub>-N standards
2. Calibrate with a matching concentration range of NO<sub>2</sub>-N standards
3. The column efficiency is determined by the equation:

$$\frac{S_{\text{NO}_3\text{-N}}}{S_{\text{NO}_2\text{-N}}} \times 100 = E$$

$S_{\text{NO}_3\text{-N}}$  = slope of NO<sub>3</sub> calibration

$S_{\text{NO}_2\text{-N}}$  = slope of NO<sub>2</sub> calibration

E = % efficiency

4. If the efficiency is < 90%, the column should be repacked

**Concentration Ratio Method**

1. Calibrate with the mid-range NO<sub>2</sub>-N or NO<sub>3</sub>-N standards
2. Run a known concentration NO<sub>2</sub>-N standard
3. Run a matching concentration NO<sub>3</sub>-N standard
4. The column efficiency is determined by the equation:

$$\frac{C_{\text{NO}_3\text{-N}}}{C_{\text{NO}_2\text{-N}}} \times 100 = E$$

$C_{\text{NO}_3\text{-N}}$  = concentration of NO<sub>3</sub> standard

$C_{\text{NO}_2\text{-N}}$  = concentration of NO<sub>2</sub> standard

E = % efficiency

5. If the efficiency is < 90%, the column should be repacked



**-- Timing --**

Sample Throughput: 90 samples/h; 40 s/sample

Pump Speed: 35

Cycle Period: 40 s

Nitrate + Nitrite:

Inject to Start of Peak Period: 22 s

Inject to End of Peak Period: 60 s

Nitrite (no column):

Inject to Start of Peak Period: 15 s

**QC8000 Settings:****Analyte data:**

Peak Base Width: 29 s

% Width Tolerance: 100

Threshold: 3000

Inject to Peak Start: 22 s

**Calibration Data:**

Calibration Fit Type: 1st Order Polynomial

Weighting Method: 1/X

**Sampler Timing:**

Min. Probe in Wash Period: 9 s

Probe in Sample Period: 20 s

**Valve Timing:**

Load Period: 20 s



Inject Period: 20 s

**QuikChem AE Settings:**

**Parameter, Data Window:**

Top Scale Response: approx. 1.0 abs

Bottom Scale Response : 0.00 abs

**Series 4000/System IV Settings:**

Gain = 140 x 1

**-- System Notes --**

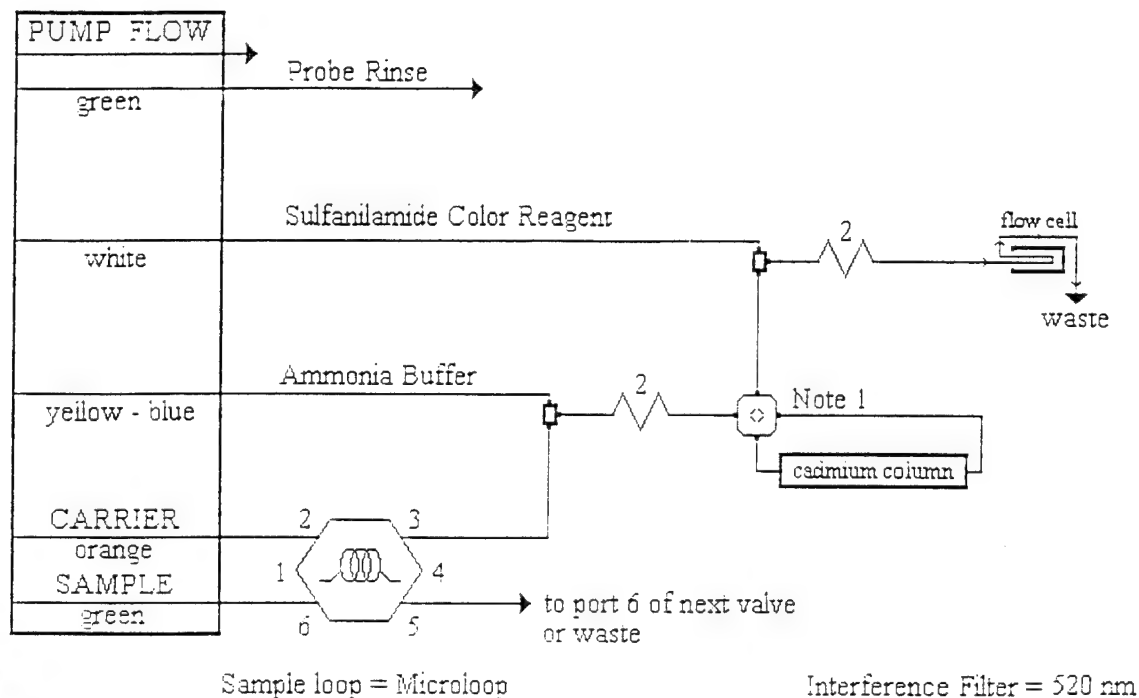
QuikChem AE: The  $\text{NO}_3^-$  concentration can be determined in an RDF using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

SERIES 4000/SYSTEM IV: The  $\text{NO}_3^-$  concentration can be determined in a pseudo-channel using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

**-- Sources --**

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 353.2
2. **Methods for Determination of Inorganic Substances in Water and Fluvial Sediments**. Book 5. Chapter A1. U.S. Department of the Interior. U.S. Geological Survey



**NITRATE/NITRITE, NITRITE MANIFOLD DIAGRAM:**

**CARRIER** is water.

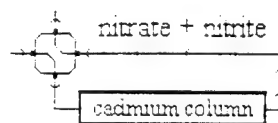
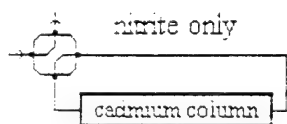
2 is 135 cm of tubing on a 2 inch coil support

**APPARATUS:** Standard valve, flow cell, and detector head modules are used.

All manifold tubing is **0.8 mm (0.032 in)** i.d. This is 5.2 uL/cm.

**Notes:**

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



**MANIFOLD DIAGRAM REVISION DATE:** 11Aug94 L. Carlson



Statistical analysis of data for NPDES EPA Equivalency, 17 Dec. 86  
 Number of calibration (x,y) pairs is 20

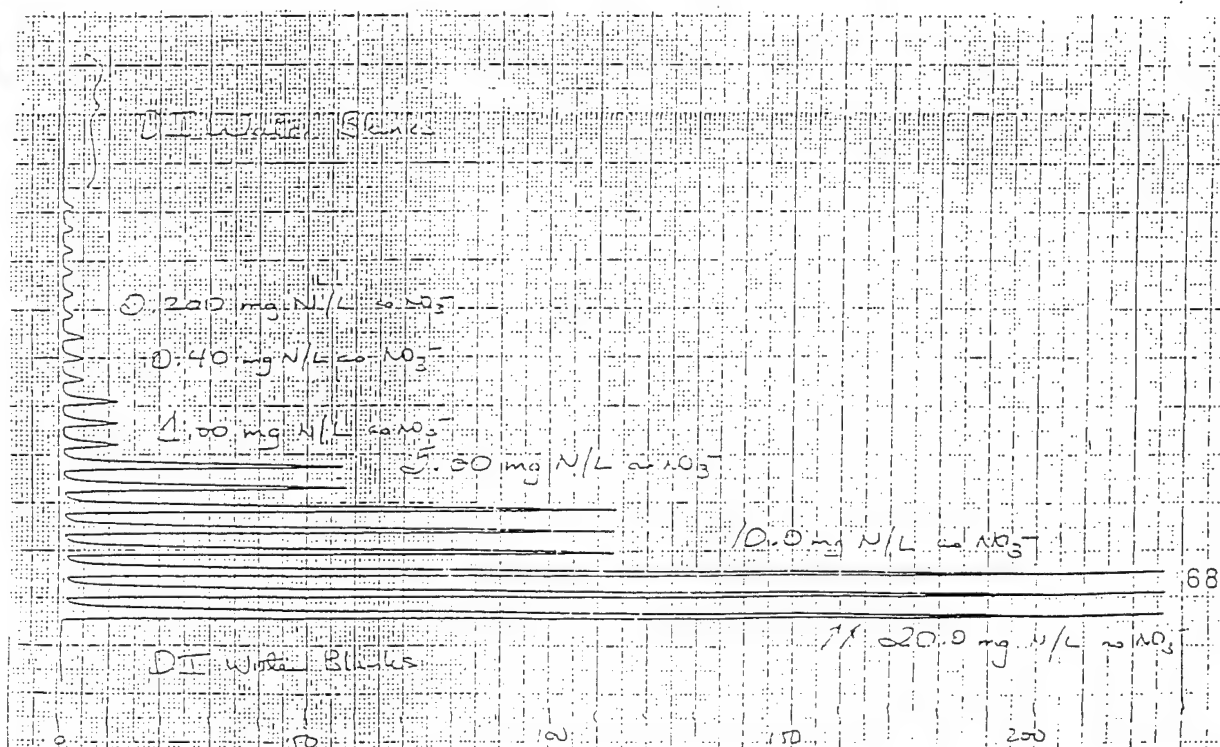
(x,y) no. 1 = 20 , 225  
 (x,y) no. 2 = 20 , 225.5  
 (x,y) no. 3 = 20 , 225.5  
 (x,y) no. 4 = 10 , 112.5  
 (x,y) no. 5 = 10 , 112.5  
 (x,y) no. 6 = 10 , 112.5  
 (x,y) no. 7 = 5 , 56.5  
 (x,y) no. 8 = 5 , 56.5  
 (x,y) no. 9 = 1 , 11  
 (x,y) no. 10 = 1 , 11  
 (x,y) no. 11 = 1 , 10.5  
 (x,y) no. 12 = .4 , 4  
 (x,y) no. 13 = .4 , 4  
 (x,y) no. 14 = .4 , 4  
 (x,y) no. 15 = .2 , 2  
 (x,y) no. 16 = .2 , 2  
 (x,y) no. 17 = .2 , 2  
 (x,y) no. 18 = .2 , 2  
 (x,y) no. 19 = .2 , 2  
 (x,y) no. 20 = .2 , 2

The best fit equation is

$$y = 11.2849 x + (-.3214)$$

The correlation coefficient is .99999

The sd in the slope is .0069 , or .06 %RSD.





QuikChem Method 10-107-04-1-C

## **Nitrate/Nitrite, Nitrite in Surface Water, Wastewater**

0.02 to 2.0 mg N/L as  $\text{NO}_3^-$  or  $\text{NO}_2^-$

### **-- Principle --**

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone also can be determined by removing the cadmium column.

### **-- Interferences --**

1. Residual chlorine can interfere by oxidizing the cadmium column.
2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.
3. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.
4. Sample turbidity may interfere. Remove turbidity first by filtration with 0.45  $\mu\text{m}$  pore diameter membrane filter prior to analysis.

### **-- Special Apparatus --**

1. Cadmium-Copper Reduction Column (Lachat Part # 50237)

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### -- Sample Handling and Preservation --

Nitrite will be oxidized by air  $O_2$  to nitrate in a few days. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at  $4^{\circ}C$ . When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc.  $H_2SO_4$  per liter) and refrigerated. **CAUTION:** Samples must not be preserved with mercuric chloride or thiosulfate because this will degrade the cadmium column.

If build-up of suspended matter in the reduction column restricts sample flow, the samples may be pre-filtered.

### -- Preparation of Reagents --

Use deionized water (10 megohm) for all solutions.

#### Reagent 1. 15 N Sodium hydroxide

Add **150 g NaOH** very slowly to **250 mL of water**. **CAUTION:** The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

#### Reagent 2. Ammonium chloride buffer, pH 8.5

**By Volume:** In a 1 L volumetric flask, dissolve **85.0 g ammonium chloride** ( $NH_4Cl$ ) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ( $Na_2EDTA \cdot 2H_2O$ ) in about **800 mL water**. Adjust the pH to 8.5 with **15 N sodium hydroxide**. Dilute to the mark and invert to mix.

**By Weight:** To a tared 1 L container, add **85.0 g ammonium chloride** ( $NH_4Cl$ ), **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ( $Na_2EDTA \cdot 2H_2O$ ) and **938 g water**. Shake or stir until dissolved. Then adjust the pH to 8.5 with **15 N sodium hydroxide**.

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination.. An alternative recipe for the ammonium chloride buffer is

**CAUTION:** Fumes!

**By Volume:** In a hood, to a 1 L volumetric flask add **500 mL water**, **105 mL concentrated hydrochloric acid** ( $HCl$ ), **95 mL ammonium hydroxide** ( $NH_4OH$ ), and **1.0 g disodium EDTA**. Dissolve and dilute to the mark. Invert to mix. Adjust pH to 8.5 with  $HCl$  or 15 N  $NaOH$ .

**By Weight:** In a hood, to a tared 1 L container add **865 g water**, **126 g concentrated hydrochloric acid** ( $HCl$ ), **85 g ammonium hydroxide** ( $NH_4OH$ ) and **1.0 g disodium EDTA**. Stir until dissolved. Adjust pH to 8.5 with  $HCl$  or 15 N  $NaOH$ .



### Reagent 3. Sulfanilamide color reagent

**By Volume:** To a 1 L volumetric flask add about 600 mL water. Then add 100 mL of 85% phosphoric acid ( $\text{H}_3\text{PO}_4$ ), 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet, and stir to dissolve for 30 min. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

**By Weight:** To a tared, dark 1 L container add 876 g water, 170 g 85% phosphoric acid ( $\text{H}_3\text{PO}_4$ ), 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Shake until wetted and stir with stir bar for 30 min. dissolved. This solution is stable for one month.

### -- Preparation of Standards --

**NOTE:** Following are standards preparations for a 1 channel system determining  $\text{NO}_2^- + \text{NO}_3^-$  or  $\text{NO}_2^-$  and a 2 channel system where one channel is used for  $\text{NO}_2^- + \text{NO}_3^-$  and the other channel is used for determining  $\text{NO}_2^-$ . For the 1 channel system, either  $\text{NO}_2^-$  or  $\text{NO}_3^-$  standards may be used. We recommend the use of  $\text{NO}_3^-$  standards when running a 1 channel method for  $\text{NO}_2^- + \text{NO}_3^-$ . For the 2 channel system, we recommend the use of both  $\text{NO}_2^- + \text{NO}_3^-$  standard sets.

#### Standard 1. Stock Nitrate Standard 2000 mg N/L as $\text{NO}_3^-$

In a 1 L volumetric flask dissolve 14.44 g potassium nitrate ( $\text{KNO}_3$ ) in about 600 mL water. Add 2 mL chloroform. Dilute to the mark and invert to mix. This solution is stable for six months.

#### Standard 2. Stock Nitrite Standard 2000.0 mg N/L as $\text{NO}_2^-$

**By Volume:** In a 1 L volumetric flask dissolve 9.86 g sodium nitrite ( $\text{NaNO}_2$ ) or 12.14 g potassium nitrite ( $\text{KNO}_2$ ) in approximately 800 mL water. Add 2 mL chloroform. Dilute to the mark and invert to mix. Refrigerate.

#### Standard 3. Working Nitrate Standard, 20.00 mg N/L as $\text{NO}_3^-$

**By Volume:** In a 1 L volumetric flask, add 10.00 mL Stock Standard 1. Dilute to the mark with water and invert to mix.

**By Weight:** To a tared 1 L container, add about 10 g Stock Standard 1. Measure the exact weight and divide by 0.01. This will give you the total weight of this diluted solution to be made. Make up this solution to the total weight with water, using a wash bottle for the last 10 g or so. Shake or stir before using.



**Standard 4. Working Nitrite Standard, 20.00 mg N/L as NO<sub>2</sub><sup>-</sup>**

**By Volume:** In a 1 L volumetric flask, add 10.00 mL Standard 2. Dilute to the mark with **water** and invert to mix.

**By Weight:** To a tared 1 L container, add about 10 g Standard 2. Measure the exact weight of this solution and divide by 0.01. This will give you the total weight of this diluted solution to be made. Make up this solution to the total weight with **water**, using a wash bottle for the last 10 g or so. Shake or stir before using.

**Set of Six Working NO<sub>3</sub><sup>-</sup> Standards:**

2.00 0.800 0.400 0.100 0.0400 0.0200 mg N/L

**By Volume:** To six 250 mL volumetric flasks add, respectively,

25.00 10.00 5.00 1.250 0.500 0.250 mL

**Working Standard 3.** Dilute each to the mark with **water** and invert to mix.

**By Weight:** To six 250 mL containers add, respectively, about

25 10 5 1.25 0.500 0.25 g

**Working Standard 3.** Take the exact weight obtained for each and divide by

0.1 0.04 0.02 0.005 0.002 0.001

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

**Set of Six Working NO<sub>2</sub><sup>-</sup> Standards:**

2.00 0.800 0.400 0.100 0.0400 0.0200 mg N/L

**By Volume:** To six 250 mL volumetric flasks add, respectively,

25.00 10.00 5.00 1.250 0.500 0.250 mL

**Working Standard 4.** Dilute each to the mark with **water** and invert to mix.



**By Weight:** To six **250 mL** containers add, respectively, about

**25    10    5    1.25   0.500   0.25   g**

of the **Working Standard 4**. Take the exact weight obtained for each and divide by

**0.1   0.04   0.02   0.005   0.002   0.001**

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.



## -- Preparation of Reagents for Cadmium Reduction Column --

### Reagent A. 1 N Hydrochloric Acid (HCl)

**By Volume:** In a 100 mL container, add 8 mL concentrated HCl to 92 mL water. Stir or shake to mix.

**By Weight:** To a 100 mL container, add 92 g water then add 9.6 g concentrated HCl. Stir or shake to mix.

### Reagent B. 2% Copper Sulfate Solution

**By Volume:** In a 1 L volumetric flask, dissolve 20 g coppersulfate( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in about 800 mL water. Dilute to mark with water. Invert to mix thoroughly.

**By Weight:** To a 1 L container, add 20 g copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to 991g water. Stir or shake to dissolve.

## -- Preparation of Cadmium Reduction Column --

### Cadmium Preparation

Place 10-20 g of coarse cadmium granules (0.3 - 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with 50 mL of acetone, then water, then two 50 mL portions of 1 N hydrochloric acid (Reagent A). Rinse several times with water. **CAUTION:** Collect and store all waste cadmium. **Cadmium is toxic and carcinogenic.** Wear gloves and follow the precautions described on the Material Safety Data Sheet.

### Copperization

Add a 100 mL portion of 2% Copper Sulfate Solution (Reagent B) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh 100 mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (Reagent 2) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a stoppered bottle under ammonium chloride buffer (Reagent 2).



## **Packing the Column**

The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that your hands are free. Unscrew one of the colored fittings from an end of the column, and pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting up higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer (Reagent 2).

Scoop up prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with DI water.

If air remains in the column or is introduced accidentally, connect the column into the manifold, turn the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

### **-- Cadmium Column Insertion Procedure --**

- a. Before inserting the column, pump all reagents into manifold.
- b. Turn the pump off.
- c. On the column, disconnect the center tubing from one of the union connectors and immediately connect to the outlet tubing of the buffer mixing coil.
- d. Connect the open tubing on the column to the tee fitting where the color reagent is added. **DO NOT LET AIR ENTER THE COLUMN.**
- e. Return the pump to normal speed.
- f. The direction of reagent flow through the column is not relevant.



## Column Efficiency Procedure

- a. Visually inspect the column. Check for air bubbles in the column or lines, gaps in the column or any change in the cadmium surface characteristics, (cadmium granules should be dark gray).
- b. If air bubbles are present in column, connect the column into the manifold, turn the pump on maximum and tap firmly with a screwdriver handle, being careful not to break the column, working up the column until all air is removed. If air cannot be removed, the column should be repacked. The columns should never be rinsed with water. Cadmium columns should be stored filled with buffer. If air enters the column, efficiency will decrease.
- c. Check the flow efficiency by disconnecting the cadmium column from the manifold and reconnecting to a green pump tube. Pump buffer through the packed column and collect in a graduated cylinder. The flow rate with the column connected should be greater than 4.0 mL/min.
- d. There are two procedures for determining column efficiency, as follows:

### Slope Ratio Method

1. Calibrate with the mid-range NO<sub>3</sub>-N standards
2. Calibrate with a matching concentration range of NO<sub>2</sub>-N standards
3. The column efficiency is determined by the equation:

$$\frac{S_{NO_3-N}}{S_{NO_2-N}} \times 100 = E$$

S<sub>NO<sub>3</sub>-N</sub> = slope of NO<sub>3</sub> calibration  
S<sub>NO<sub>2</sub>-N</sub> = slope of NO<sub>2</sub> calibration  
E = % efficiency

4. If the efficiency is <90%, the column should be repacked

### Concentration Ratio Method

1. Calibrate with the mid-range NO<sub>2</sub>-N or NO<sub>3</sub>-N standards
2. Run a known concentration NO<sub>2</sub>-N standard
3. Run a matching concentration NO<sub>3</sub>-N standard
4. The column efficiency is determined by the equation:

$$\frac{C_{NO_3-N}}{C_{NO_2-N}} \times 100 = E$$

C<sub>NO<sub>3</sub>-N</sub> = concentration of NO<sub>3</sub> standard  
C<sub>NO<sub>2</sub>-N</sub> = concentration of NO<sub>2</sub> standard  
E = % efficiency

5. If the efficiency is <90%, the column should be repacked



## -- Timing --

Sample throughput: 90 samples/h: 40 s/sample

Pump speed: 35

Cycle period: 40 s

Nitrate + Nitrite:

Inject to start of peak period: 22 s

Inject to end of peak period: 60 s

Nitrite (no column):

Inject to start of peak period: 15 s

Inject to end of peak period: 53 s

### QuikChem AE Settings:

#### Parameter, Data Window:

Top Scale Response: approx. 0.50 abs

Bottom Scale Response : 0.00 abs

### Series 4000/System IV Settings:

GAIN = 140 x 1

## -- System Notes --

QuikChem AE: The  $\text{NO}_3^-$  concentration can be determined in an RDF using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

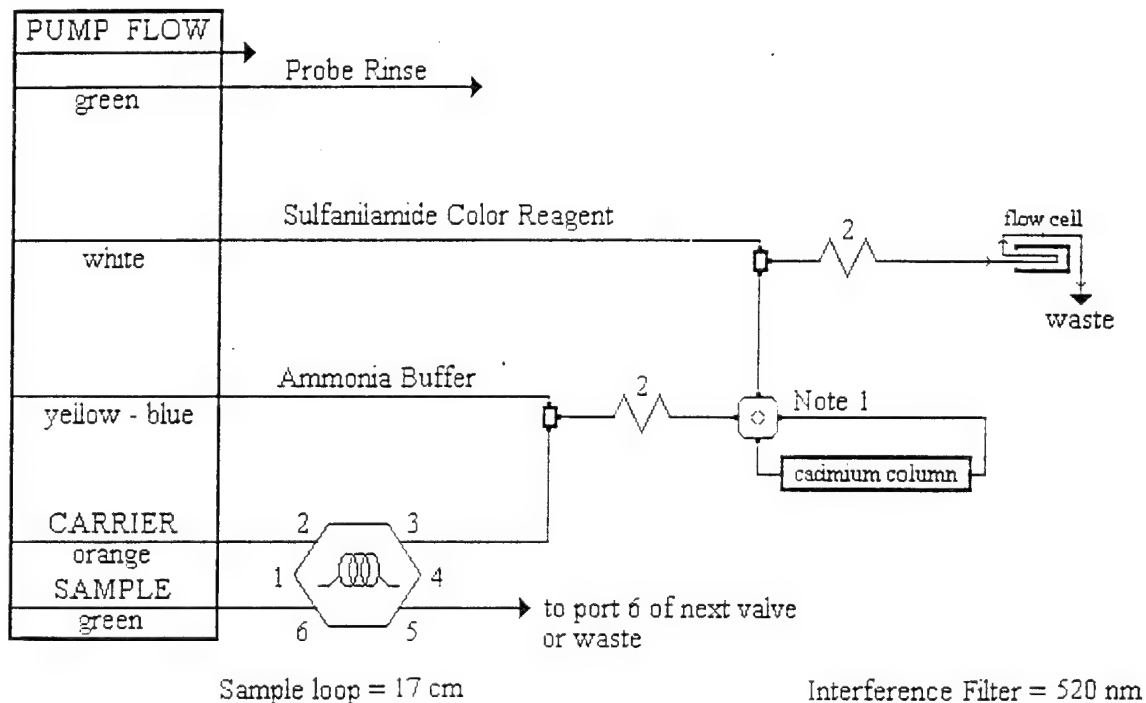
SERIES 4000/SYSTEM IV: The  $\text{NO}_3^-$  concentration can be determined in a pseudo-channel using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

## -- Sources --

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**. EPA-600/4-79-020, Revised March 1983, Method 353.2.
2. **Methods for Determination of Inorganic Substances in Water and Fluvial Sediments**. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey



# NITRATE/NITRITE, NITRITE MANIFOLD DIAGRAM:



CARRIER is water.

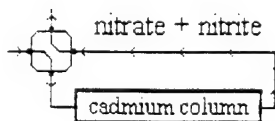
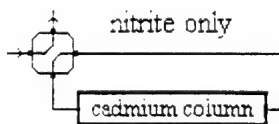
2 is 135 cm of tubing on a 2 inch coil support

APPARATUS: Standard valve, flow cell, and detector head modules are used.

All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

## Notes:

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



MANIFOLD DIAGRAM REVISION DATE: 11Aug94 L. Carlson



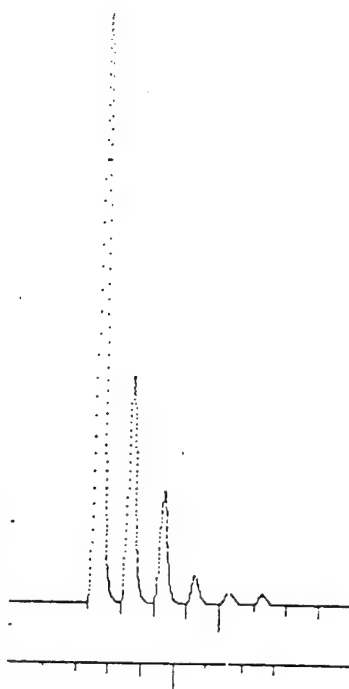
QuikChem AE Calibration Report for Calibration 92011604

Method: Nitrate Nitrogen

This calibration was done on 01/16/92 03:11 pm

This report prepared on 01/23/92 05:01 pm

Calibration 01/16/1992, 03:03 pm



1. Standard A (1)
2. Standard B (1)
3. Standard C (1)
4. Standard D (1)
5. Standard E (1)
6. Standard F (1)
7. Standard A (2)
8. Standard B (2)
9. Standard C (2)
10. Standard D (2)
11. Standard E (2)
12. Standard F (2)

		Average Concentrations			Baseline Corrected
Analyte	Units	Known	Determined	% Residual	Average Absorbance
Standard A, NITRATE	mg/L	2.000	2.010	-0.50	0.1259
		Average Concentrations			Baseline Corrected
Standard B, NITRATE	mg/L	0.800	0.700	2.54	0.0410
		Average Concentrations			Baseline Corrected
Standard C, NITRATE	mg/L	0.400	0.309	2.63	0.0243
		Average Concentrations			Baseline Corrected
Standard D, NITRATE	mg/L	0.100	0.100	-0.15	0.0060
		Average Concentrations			Baseline Corrected
Standard E, NITRATE	mg/L	0.040	0.043	-0.53	0.0024
		Average Concentrations			Baseline Corrected
Standard F, NITRATE	mg/L	0.000	0.007	100.00	0.0001

End of Calibration Report For Calibration 92011604

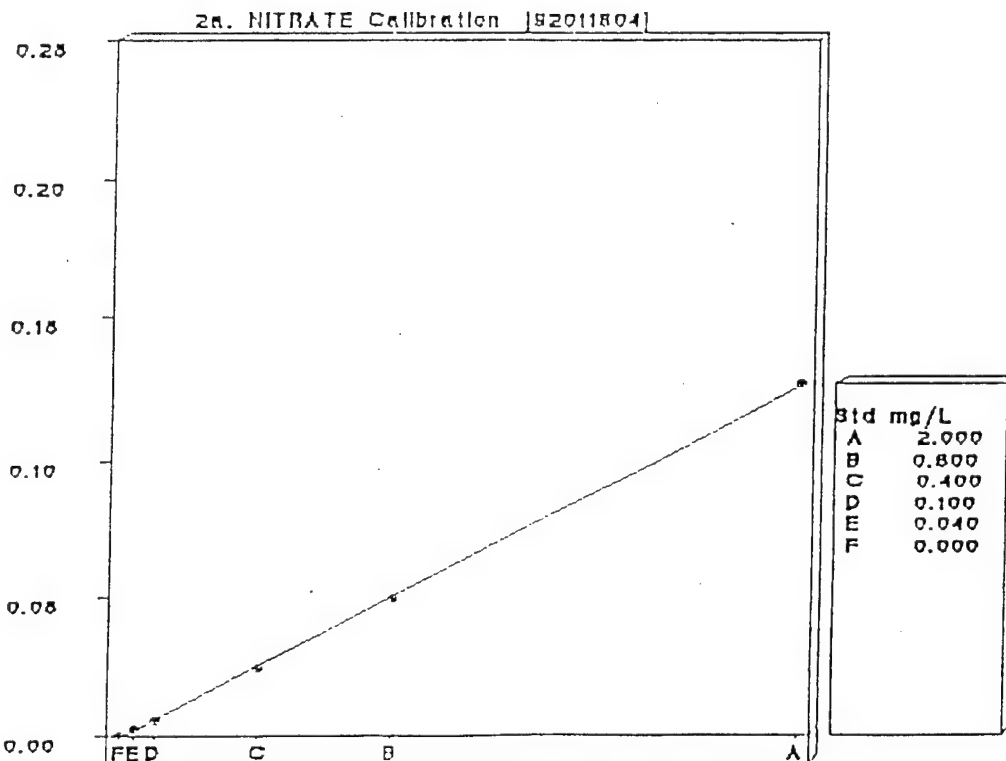
Channel: NITRATE

Correlation Coefficients

Seg Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1 A-F	0.9970	0.9973	0.9978	0.9978	0.9997	0.9925

Percent Standard Deviation in Slope

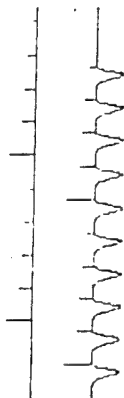
1 A-F	0.6	1.1	0.6	0.5	0.7	1.1
-------	-----	-----	-----	-----	-----	-----





Rack 2 (Ref: 92011614) 01/16/1992, 03:54 pm

281. 0.1 ug N/L (1)  
 282. 0.1 ug N/L (1)  
 283. 0.1 ug N/L (1)  
 284. 0.1 ug N/L (1)  
 285. 0.1 ug N/L (1)  
 286. 0.1 ug N/L (1)  
 287. 0.1 ug N/L (1)  
 288. 0.1 ug N/L (1)  
 289. 0.1 ug N/L (1)  
 290. 0.1 ug N/L (1)



— Calibration now in effect (92011604) was done on 01/16/92 03:11 pm

— Page 1 of report for tray 92011614.RS

MDL

Cup# Sample ID	2 NITRATE ug/L
281 0.1 ug N/L	0.182
282 0.1 ug N/L	0.079
283 0.1 ug N/L	0.183
284 0.1 ug N/L	0.182
285 0.1 ug N/L	0.180
286 0.1 ug N/L	0.181
287 0.1 ug N/L	0.181
288 0.1 ug N/L	0.182
289 0.1 ug N/L	0.079
290 0.1 ug N/L	0.182

$$\bar{X} = 0.1011$$

$$S = 0.0013$$

$$MDL = 0.004$$

Rack 2 (Ref: 92011611) 01/16/1992, 03:22 pm

281. Blank (1)  
 282. Blank (1)  
 283. Blank (1)  
 284. Blank (1)  
 285. Blank (1)  
 286. Blank (1)  
 287. Blank (1)  
 288. Blank (1)  
 289. Blank (1)  
 290. Blank (1)



— Calibration now in effect (92011604) was done on 01/16/92 03:11 pm

— Page 1 of report for tray 92011611.RS

EMDL

Cup# Sample ID	2 NITRATE ug/L
281 Blank	0.0071
282 Blank	0.0061
283 Blank	0.0061
284 Blank	0.0051
285 Blank	0.0071
286 Blank	0.0061
287 Blank	0.0061
288 Blank	0.0071
289 Blank	0.0041
290 Blank	0.0061

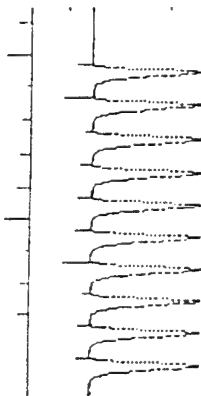
$$\bar{X} = 0.0063$$

$$S = 0.0011$$

$$EMDL = 0.0055$$

Rack 1 (Ref: 92011610) 01/16/1992, 03:13 pm

181. 0.4 ug N/L (1)  
 182. 0.4 ug N/L (1)  
 183. 0.4 ug N/L (1)  
 184. 0.4 ug N/L (1)  
 185. 0.4 ug N/L (1)  
 186. 0.4 ug N/L (1)  
 187. 0.4 ug N/L (1)  
 188. 0.4 ug N/L (1)  
 189. 0.4 ug N/L (1)  
 190. 0.4 ug N/L (1)



— Calibration now in effect (92011604) was done on 01/16/92 03:11 pm

— Page 1 of report for tray 92011610.RS

Precision

Cup# Sample ID	2 NITRATE ug/L
181 0.4 ug N/L	0.378
182 0.4 ug N/L	0.387
183 0.4 ug N/L	0.392
184 0.4 ug N/L	0.388
185 0.4 ug N/L	0.388
186 0.4 ug N/L	0.303
187 0.4 ug N/L	0.387
188 0.4 ug N/L	0.379
189 0.4 ug N/L	0.378
190 0.4 ug N/L	0.372

$$\bar{X} = 0.3895$$

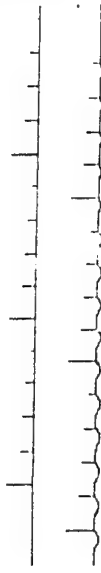
$$S = 0.0016$$

$$\%RSD = 0.40$$



Rack 3 (Ref: 92011612) 01/16/1992, 03:31 pm

381. 2.0 ug Fe/L (1)  
 382. 2.0 ug Fe/L (1)  
 383. 2.0 ug Fe/L (1)  
 384. 2.0 ug Fe/L (1)  
 385. 2.0 ug Fe/L (1)  
 386. 10 ug Fe/L (1)  
 387. 10 ug Fe/L (1)  
 388. 10 ug Fe/L (1)  
 389. 10 ug Fe/L (1)  
 390. 10 ug Fe/L (1)  
 391. 20 ug Fe/L (1)  
 392. 20 ug Fe/L (1)  
 393. 20 ug Fe/L (1)  
 394. 20 ug Fe/L (1)  
 395. 20 ug Fe/L (1)



— Calibration now in effect (92011604) was done on 01/16/92 03:11 pm

— Page 1 of report for tray 92011612.RS

Fe interference

Cup# Sample ID

2  
NITRATE  
ug/L

381 2.0 ug Fe/L  
 382 2.0 ug Fe/L  
 383 2.0 ug Fe/L  
 384 2.0 ug Fe/L  
 385 2.0 ug Fe/L  
 386 10 ug Fe/L  
 387 10 ug Fe/L  
 388 10 ug Fe/L  
 389 10 ug Fe/L  
 390 10 ug Fe/L  
 391 20 ug Fe/L  
 392 20 ug Fe/L  
 393 20 ug Fe/L  
 394 20 ug Fe/L  
 395 20 ug Fe/L

0.013  
 0.015  
 0.016  
 0.011  
 0.015  
 0.029  
 0.030  
 0.030  
 0.029  
 0.028  
 0.033  
 0.033  
 0.031  
 0.031  
 0.033

$$\bar{x} = 0.014$$

$$\bar{x} = 0.0292$$

$$\bar{x} = 0.0322$$

— Calibration now in effect (92011604) was done on 01/16/92 03:11 pm

— Page 1 of report for tray 92011615.RS

Carryover

Cup# Sample ID

2  
NITRATE  
ug/L

381 2.0 ug H/L  
 382 Blank  
 383 Blank  
 384 Blank  
 385 Blank  
 386 2.0 ug H/L  
 387 Blank  
 388 Blank  
 389 Blank  
 390 Blank

2.010  
 -0.001  
 0.006  
 0.006  
 0.005  
 2.000  
 -0.003  
 0.007  
 0.005  
 0.005

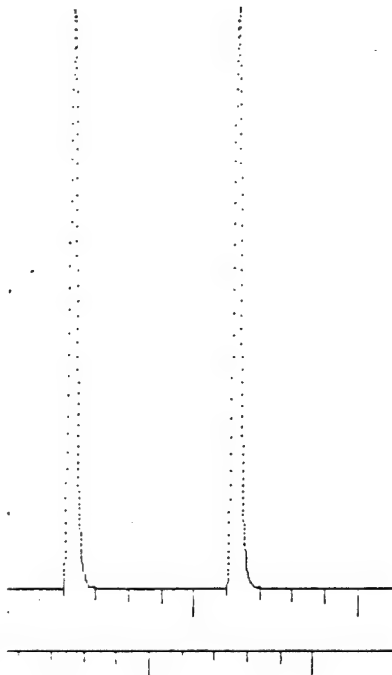
$$\bar{x} = 0.004$$

$$s = 0.0029$$

$$\%CI = 0.004 \pm 0.0053$$

Rack 3 (Ref: 92011615) 01/16/1992, 04:03 pm

381. 2.0 ug H/L (1)  
 382. Blank (1)  
 383. Blank (1)  
 384. Blank (1)  
 385. Blank (1)  
 386. 2.0 ug H/L (1)  
 387. Blank (1)  
 388. Blank (1)  
 389. Blank (1)  
 390. Blank (1)





QuikChem Method 10-107-04-1-D

## Nitrate/Nitrite, Nitrite in Surface Water, Wastewater

0.5 to 50.0 mg N/L as  $\text{NO}_3^-$  or  $\text{NO}_2^-$

### -- Principle --

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column.

### -- Interferences --

1. Residual chlorine can interfere by oxidizing the cadmium column.
2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.
3. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.
4. Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45  $\mu\text{m}$  pore diameter membrane filter prior to analysis.

### -- Special Apparatus --

1. Cadmium-Copper Reduction Column (Lachat Part # 50237)



## -- Sample Handling and Preservation --

Nitrite will be oxidized by air to nitrate in a few days. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc.  $\text{H}_2\text{SO}_4$  per liter) and refrigerated. **CAUTION:** Samples must not be preserved with mercuric chloride or thiosulfate because this will degrade the cadmium column.

If build-up of suspended matter in the reduction column restricts sample flow, the samples may be pre-filtered.

## -- Preparation of Reagents --

Use deionized water (10 megohm) for all solutions.

### Reagent 1. 15 N Sodium hydroxide

In a 1 L container, add 150 g NaOH very slowly to 250 mL of water. **CAUTION:** The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

### Reagent 2. Ammonium chloride buffer, pH 8.5

**By Volume:** In a 1 L volumetric flask, dissolve 85.0 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate ( $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ ) in about 800 mL water. Dilute to the mark and invert to mix. Adjust the pH to 8.5 with 15 N sodium hydroxide.

**By Weight:** To a tared 1 L container, add 85.0 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ), 1.0 g disodium ethylenediamine tetraacetic acid dihydrate ( $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ ) and 988 g water. Shake or stir until dissolved. Then adjust the pH to 8.5 with 15 N sodium hydroxide.

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. An alternative recipe for the ammonium chloride buffer is:

**By Volume:** **CAUTION:** Fumes!!! In a hood, to a 1 L volumetric flask add 500 mL water, 105 mL concentrated hydrochloric acid (HCl), 95 mL ammonium hydroxide ( $\text{NH}_4\text{OH}$ ), and 1.0 g disodium EDTA. Dissolve and dilute to the mark. Invert to mix. Adjust the pH to 8.5 with HCl or 15 N NaOH.

**By Weight:** **CAUTION:** Fumes!!! In a hood, to a tared 1 L container add 865 g water, 126 g concentrated hydrochloric acid (HCl), 85 g ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) and 1.0 g disodium EDTA. Stir until dissolved. Adjust the pH to 8.5 with HCl or 15 N NaOH.



**Reagent 3. Sulfanilamide color reagent**

**By Volume:** To a 1 L volumetric flask add about 600 mL water. Then add 100 mL of 85% phosphoric acid ( $\text{H}_3\text{PO}_4$ ), 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet, and stir to dissolve for 30 min. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

**By Weight:** To a tared, dark 1 L container add 876 g water, 170 g 85% phosphoric acid ( $\text{H}_3\text{PO}_4$ ), 40.0 g sulfanilamide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake until wetted and stir with stir bar for 30 min. until dissolved. This solution is stable for one month.

**-- Preparation of Standards --**

**NOTE:** Following are standards preparations for a 1 channel system determining  $\text{NO}_2^- + \text{NO}_3^-$  or  $\text{NO}_2^-$  and a 2 channel system where one channel is used for  $\text{NO}_2^- + \text{NO}_3^-$  and the other channel is used for determining  $\text{NO}_2^-$ . For the 1 channel system, either  $\text{NO}_2^-$  or  $\text{NO}_3^-$  standards may be used. We recommend the use of  $\text{NO}_3^-$  standards when running a 1 channel method for  $\text{NO}_2^- + \text{NO}_3^-$ . For the 2 channel system, we recommend the use of both  $\text{NO}_2^- + \text{NO}_3^-$  standard sets.

**Standard 1. Stock Nitrate Standard 500.0 mg N/L as  $\text{NO}_3^-$** 

In a 1 L volumetric flask dissolve 3.61 g potassium nitrate ( $\text{KNO}_3$ ) in about 600 mL water. Add 2 mL chloroform. Dilute to the mark and invert to mix. This solution is stable for six months.

**Standard 2. Stock Nitrite Standard 500.0 mg N/L as  $\text{NO}_2^-$** 

In a 1 L volumetric flask dissolve 2.465 g sodium nitrite ( $\text{NaNO}_2$ ) or 3.035 g potassium nitrite ( $\text{KNO}_2$ ) in approximately 800 mL water. Add 2 mL chloroform. Dilute to the mark and invert to mix. Refrigerate.

**Set of Six Working  $\text{NO}_2^-$  Standards:**

50.0   20.0   10.0   2.50   1.00   0.50   mg N/L

**By Volume:** To six 250 mL volumetric flasks add, respectively,

25.0   10.0   5.00   1.25   0.50   0.25   mL

of the Working Stock Standard 2. Dilute each to the mark with water and invert to mix.



**By Weight:** To six 250 mL containers add, respectively, about

25    10    5    1.25    0.50    0.25    g

of the **Working Stock Standard 2**. Take the exact weight obtained for each and divide by

0.1    0.04    0.02    0.005    0.002    0.001

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

**Set of Six Working NO<sub>3</sub><sup>-</sup> Standards:**

50.0    20.0    10.0    2.50    1.00    0.50    mg N/L

**By Volume:** To six 250 mL volumetric flasks add, respectively,

25.0    10.0    5.00    1.25    0.50    0.25    mL

of the **Working Stock Standard 1**. Dilute each to the mark with **water** and invert to mix.

**By Weight:** To six 250 mL containers add, respectively, about

25    10    5    1.25    0.50    0.25    g

of the **Working Stock Standard 1**. Take the exact weight obtained for each and divide by

0.1    0.04    0.02    0.005    0.002    0.001

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.



## -- Preparation of Reagents for Cadmium Reduction Column --

### Reagent A. 1 N Hydrochloric Acid (HCl)

**By Volume:** In a 100 mL container, add 8 mL concentrated HCl to 92 mL water. Stir or shake to mix.

**By Weight:** To a 100 mL container, add 92 g water then add 9.6 g concentrated HCl. Stir or shake to mix.

### Reagent B. 2% Copper Sulfate Solution

**By Volume:** In a 1 L volumetric flask, dissolve 20 g copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in about 800 mL water. Dilute to mark with water. Invert to mix thoroughly.

**By Weight:** To a 1 L container, add 20 g copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to 991 g water. Stir or shake to dissolve.

## -- Preparation of Cadmium Reduction Column --

### Cadmium Preparation

Place 10-20 g of coarse cadmium granules (0.3 - 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with 50 mL of acetone, then water, then two 50 mL portions of 1 N hydrochloric acid (Reagent A). Rinse several times with water. **CAUTION:** Collect and store all waste cadmium. **Cadmium is toxic and carcinogenic.** Wear gloves and follow the precautions described on the Material Safety Data Sheet.

### Copperization

Add a 100 mL portion of 2% Copper Sulfate Solution (Reagent B) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh 100 mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (Reagent 2) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a stoppered bottle under ammonium chloride buffer (Reagent 2).



## Packing the Column

The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that your hands are free. Unscrew one of the colored fittings from an end of the column, and pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting up higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer (Reagent 2).

Scoop up prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with DI water.

If air remains in the column or is introduced accidentally, connect the column into the manifold, turn the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

## -- Cadmium Column Insertion Procedure --

- a. Before inserting the column, pump all reagents into manifold.
- b. Turn the pump off.
- c. On the column, disconnect the center tubing from one of the union connectors and immediately connect to the outlet tubing of the buffer mixing coil.
- d. Connect the open tubing on the column to the tee fitting where the color reagent is added. **DO NOT LET AIR ENTER THE COLUMN.**
- e. Return the pump to normal speed.
- f. The direction of reagent flow through the column is not relevant.



## Column Efficiency Procedure

- a. Visually inspect the column. Check for air bubbles in the column or lines, gaps in the column or any change in the cadmium surface characteristics, (cadmium granules should be dark gray).
- b. If air bubbles are present in column, connect the column into the manifold, turn the pump on maximum and tap firmly with a screwdriver handle, being careful not to break the column, working up the column until all air is removed. If air cannot be removed, the column should be repacked. Cadmium columns should be stored filled with buffer. If air enters the column, efficiency will decrease.
- c. Check the flow efficiency by disconnecting the cadmium column from the manifold and reconnecting to a green pump tube. Pump buffer through the packed column and collect in a graduated cylinder. The flow rate with the column connected should be greater than 4.0 mL/min.
- d. There are two procedures for determining column efficiency, as follows:

### Slope Ratio Method

1. Calibrate with the mid-range  $\text{NO}_3\text{-N}$  standards
2. Calibrate with a matching concentration range of  $\text{NO}_2\text{-N}$  standards
3. The column efficiency is determined by the equation:

$$\frac{S_{\text{NO}_3\text{-N}}}{S_{\text{NO}_2\text{-N}}} \times 100 = E$$

$S_{\text{NO}_3\text{-N}}$  = slope of  $\text{NO}_3$  calibration

$S_{\text{NO}_2\text{-N}}$  = slope of  $\text{NO}_2$  calibration

E = % efficiency

4. If the efficiency is <90%, the column should be repacked

### Concentration Ratio Method

1. Calibrate with the mid-range  $\text{NO}_2\text{-N}$  or  $\text{NO}_3\text{-N}$  standards
2. Run a known concentration  $\text{NO}_2\text{-N}$  standard
3. Run a matching concentration  $\text{NO}_3\text{-N}$  standard
4. The column efficiency is determined by the equation:

$$\frac{C_{\text{NO}_3\text{-N}}}{C_{\text{NO}_2\text{-N}}} \times 100 = E$$

$C_{\text{NO}_3\text{-N}}$  = concentration of  $\text{NO}_3$  standard

$C_{\text{NO}_2\text{-N}}$  = concentration of  $\text{NO}_2$  standard

E = % efficiency

5. If the efficiency is <90%, the column should be repacked



1. Calibrate with the mid-range  $\text{NO}_2\text{-N}$  or  $\text{NO}_3\text{-N}$  standards
2. Run a known concentration  $\text{NO}_2\text{-N}$  standard
3. Run a matching concentration  $\text{NO}_3\text{-N}$  standard
4. The column efficiency is determined by the equation:

$$\frac{C_{\text{NO}_3\text{-N}}}{C_{\text{NO}_2\text{-N}}} \times 100 = E$$

$C_{\text{NO}_3\text{-N}}$  = concentration of  $\text{NO}_3$  standard

$C_{\text{NO}_2\text{-N}}$  = concentration of  $\text{NO}_2$  standard

E = % efficiency

5. If the efficiency is <90%, the column should be repacked

### -- Timing --

Sample throughput: 90 samples/h; 40 s/sample  
Pump speed: 35

Cycle period: 40 s

Nitrate + Nitrite:

Inject to start of peak period: 45 s

Inject to end of peak period: 81 s

Nitrite (no column):

Inject to start of peak period: 33 s

Inject to end of peak period: 69 s

### QuikChem AE Settings:

### Series 4000/System IV Settings:

Gain = 090 x 1

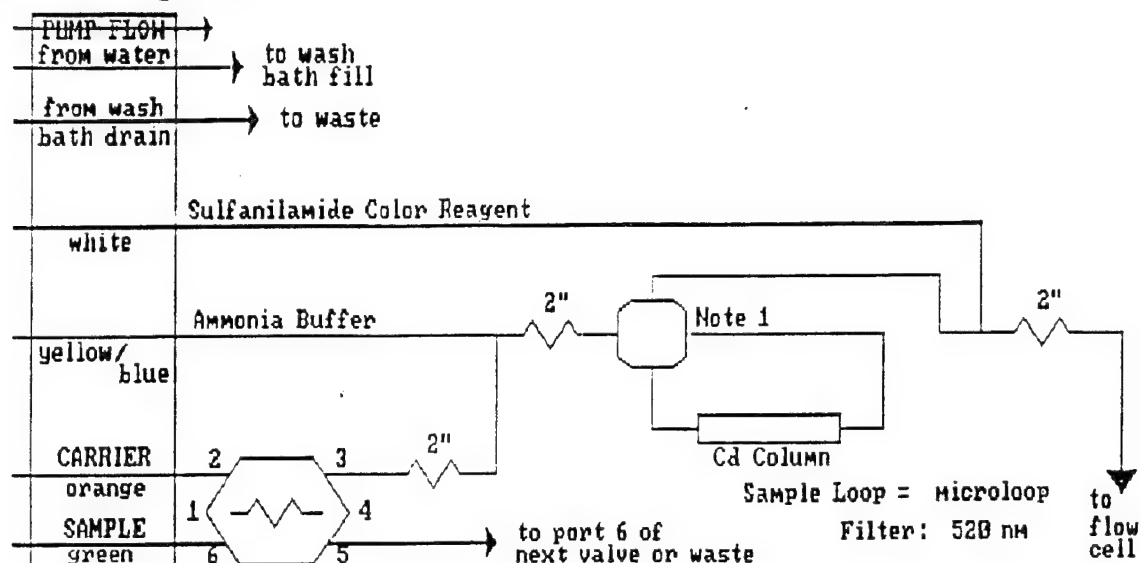
### -- System Notes --

QuikChem AE: The  $\text{NO}_3^-$  concentration can be determined in an RDF using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

SERIES 4000/SYSTEM IV: The  $\text{NO}_3^-$  concentration can be determined in a pseudo-channel using the equation  $([\text{NO}_2^- + \text{NO}_3^-] - [\text{NO}_2^-])/\text{column efficiency}$ .

### -- Sources --



**Manifold Diagram:**

CARRIER is water.

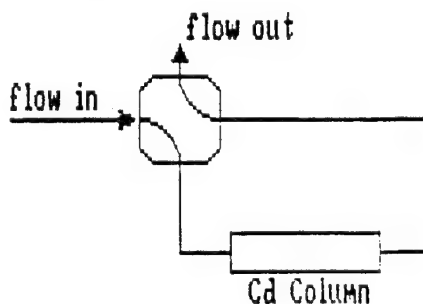
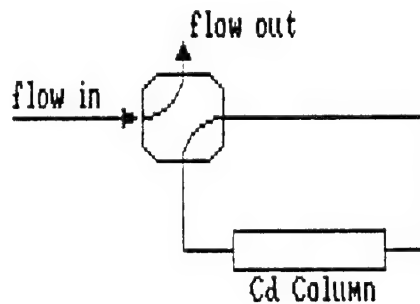
2" is 135 cm of tubing on a 2 in coil support

APPARATUS: Standard valve, flow cell, and detector head modules are used.

All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 uL/cm.

**Notes:**

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.

**STATE 1: Nitrate + Nitrite****STATE 2: Nitrite only**

MANIFOLD DIAGRAM REVISION DATE: 6 November 1992 AS



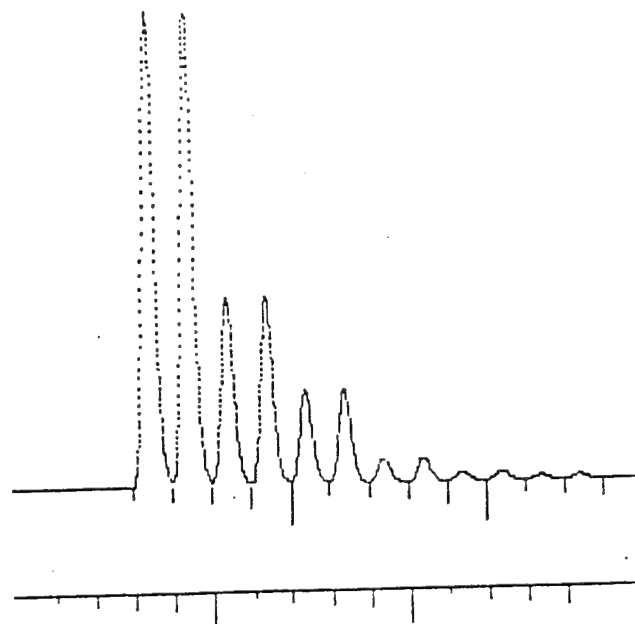
## SYSTEM NOTE:

QuikChem AE Calibration Report for Tray Reference 91072601.RC

This calibration was done on 07/26/91 at 10:44 am

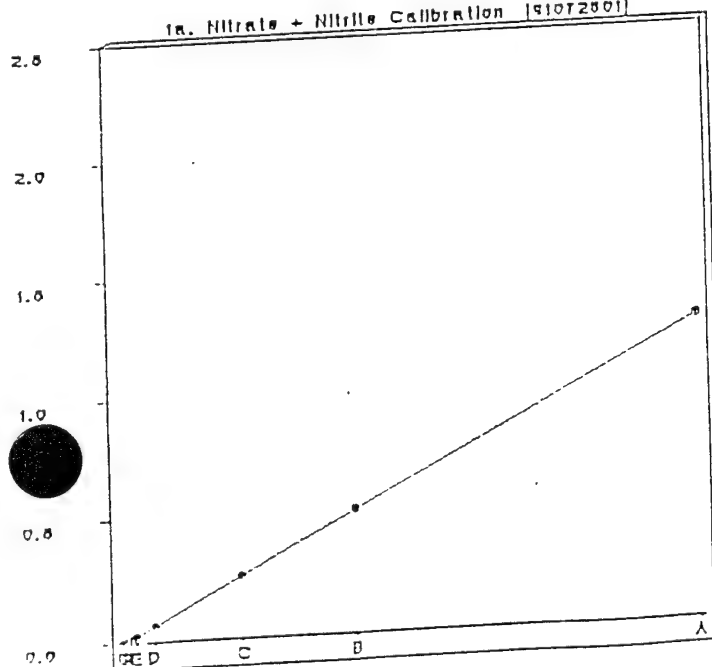
	Units	Concentration Known	Concentration Determined	% Residual	Absorbance
Standard A	mg N/L	50.000	49.980	0.04	1.2552 (Average)
		Rep. # 1	49.737	0.53	
		Rep. # 2	50.223	-0.45	
Standard B	mg N/L	20.000	20.042	-0.21	0.5032 (Average)
		Rep. # 1	19.916	0.42	
		Rep. # 2	20.168	-0.84	
Standard C	mg N/L	10.000	10.028	-0.28	0.2517 (Average)
		Rep. # 1	9.960	0.40	
		Rep. # 2	10.095	-0.95	
Standard D	mg N/L	2.500	2.450	1.99	0.0614 (Average)
		Rep. # 1	2.404	3.85	
		Rep. # 2	2.497	0.12	
Standard E	mg N/L	1.000	1.007	-0.75	0.0247 (Average)
		Rep. # 1	0.995	0.50	
		Rep. # 2	1.020	-2.00	
Standard F	mg N/L	0.500	0.502	-0.39	0.0123 (Average)
		Rep. # 1	0.502	-0.47	
		Rep. # 2	0.502	-0.32	
Standard G	mg N/L	0.000	-0.006	111.11	-0.0002 (Average)
		Rep. # 1	-0.008	111.11	
		Rep. # 2	-0.004	111.11	

End of Calibration Report

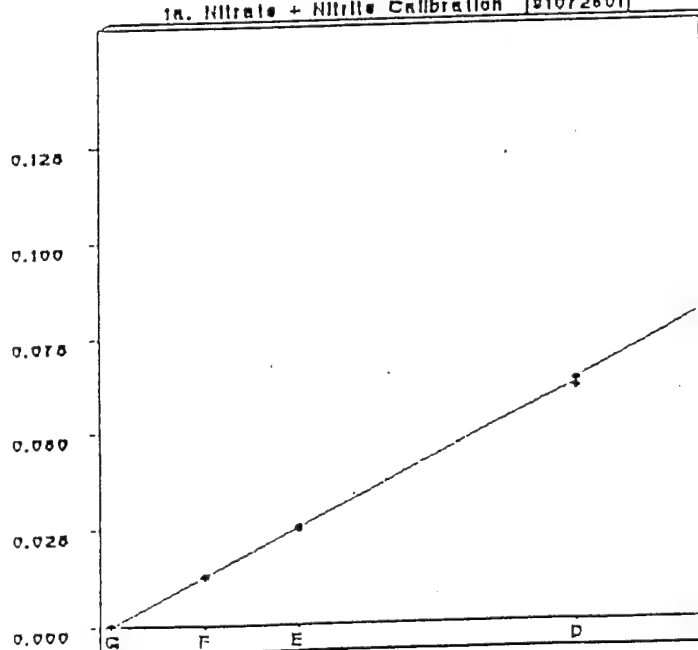


1. Standard A [1]
1. Standard A [2]
2. Standard B [1]
2. Standard B [2]
3. Standard C [1]
3. Standard C [2]
4. Standard D [1]
4. Standard D [2]
5. Standard E [1]
5. Standard E [2]
6. Standard F [1]
6. Standard F [2]
7. Standard G [1]
7. Standard G [2]

1a. Nitrate + Nitrite Calibration [91072601]



1a. Nitrate + Nitrite Calibration [91072601]





QuikCalc II Sample Report

1/31/1989 16:41

Reference: 8901310F

Operator:

ay ID:  
ethod: Nitrate

ay Comments:

Analyte -> Nitrate  
ase Subtraction Yes  
Technique Area

----- Calibration -----

Seq 1 C.C. 0.99894 .  
Seq 2 C.C. 0.99992 .

Calibration Statistics For Channel 1: Nitrate

Seq: 1 Slope: 28.29106, Intercept: 33.020, CC: 0.9989 Rspns Lim: 174

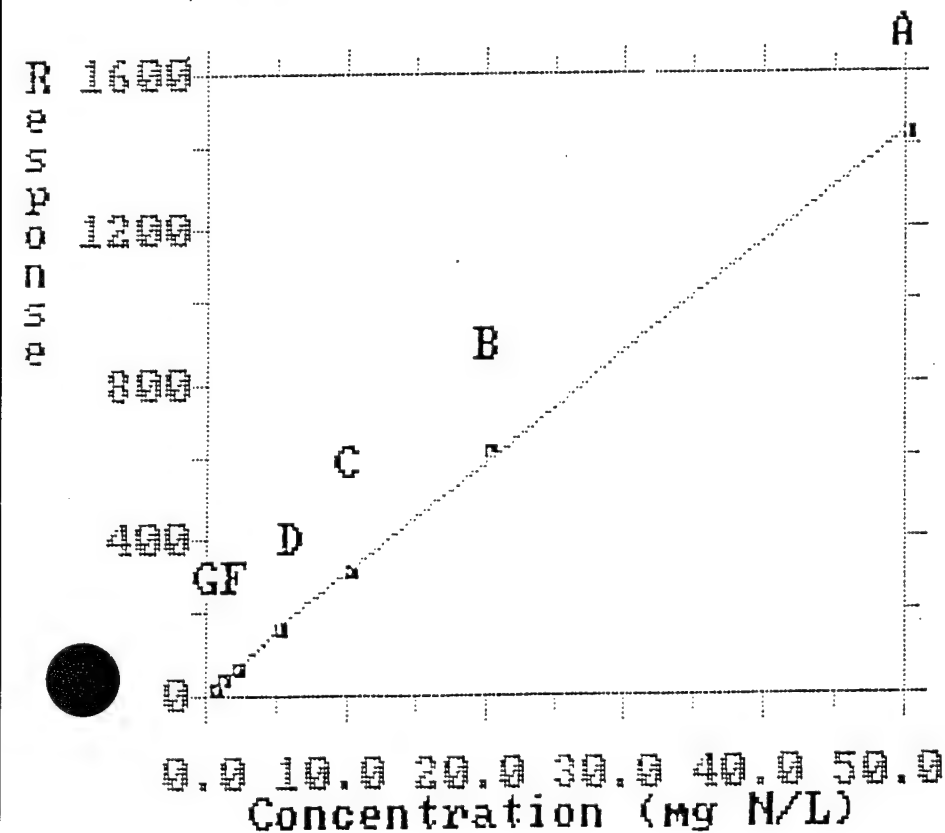
Std	Ave Rspns	Conc	Derived	%Res	Rspns 1	Rspns 2	Rspns 3
A	1439	50.000	49.700	0.6	1457	1427	1433
B	622	20.000	20.807	-4.0	612	625	628
C	317	10.000	10.021	-0.2	314	317	319
D	160	5.000	4.472	10.6	159	160	160

Seq: 2 Slope: 31.96705, Intercept: -0.311, CC: 0.9999 Rspns Lim: 9999999

Std	Ave Rspns	Conc	Derived	%Res	Rspns 1	Rspns 2	Rspns 3
D	160	5.000	5.000	-0.0	159	160	160
E	64	2.000	2.000	0.0	63	64	64
F	32	1.000	0.996	0.4	31	32	32
G	16	0.500	0.504	-0.9	16	16	16

Press [N] if you want to delete [undelete] a data point, any key otherwise.

## 1. Nitrate



11-31-89

Helena

10-107-04-1-D

0.5 to 50 mg/L

520

Flow Cell: 10mm Glass Quartz

Temp: 30.8

Conc. Unit: 1

Time Scale: 10.95

Pump Speed: 35

Temperature: 20.0

Sample Loop: 1000

Cycle Time: 40 sec

Conc. Unit: 27.00

30 mg/L

20 mg N/L

10 mg N/L

5 mg N/L

2 mg N/L

1 mg N/L

0.5 mg N/L

0.2 mg N/L

0.1 mg N/L

0.05 mg N/L

0.02 mg N/L

0.01 mg N/L

0.005 mg N/L

0.002 mg N/L

0.001 mg N/L

0.0005 mg N/L

0.0002 mg N/L

0.0001 mg N/L

0.00005 mg N/L

0.00002 mg N/L

0.00001 mg N/L

0.000005 mg N/L

0.000002 mg N/L

0.000001 mg N/L

0.0000005 mg N/L

0.0000002 mg N/L

0.0000001 mg N/L

0.00000005 mg N/L

0.00000002 mg N/L

0.00000001 mg N/L

0.000000005 mg N/L



# Technicon AutoAnalyzer II



INDUSTRIAL METHOD No. 100-70W/B

Released: SEPTEMBER 1973 / Revised: JANUARY 1978

## NITRATE AND NITRITE IN WATER AND WASTEWATER (RANGE: 0.04-2.0 mg N/l)

### GENERAL DESCRIPTION

This automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column.<sup>1,2</sup> The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

In surface waters normally encountered in surveillance studies, the concentration of oxidizing or reducing agents and potentially interfering metal ions are well below the limits causing interferences. When present in sufficient concentration, metal ions are well below the limits causing interferences. When present in sufficient concentration, metal ions may produce a positive error, i.e., divalent mercury and divalent copper may form colored complex ions having absorption bands in the region of color measurement.<sup>3</sup> High concentrations of such reducing substances as sulfite or sulfide will adversely affect column operation.

### PERFORMANCE AT 40 SAMPLES PER HOUR

#### USING AQUEOUS STANDARDS

Sensitivity at 2.0 mg N/l 0.72 absorbance units

Coefficient of Variation

at 1.0 mg N/l  $\pm 0.31$  %

Detection Limit 0.04 mg /Nl

### REAGENTS

Unless otherwise specified, all chemicals should be of ACS grade or equivalent.

### LIST OF RAW MATERIALS

- Ammonium Chloride ( $\text{NH}_4\text{Cl}$ )
- Ammonium Hydroxide ( $\text{NH}_4\text{OH}$ )
- Sulfanilamide ( $\text{C}_6\text{H}_5\text{N}_2\text{O}_2\text{S}$ )
- Phosphoric Acid, concentrated ( $\text{H}_3\text{PO}_4$ )
- N-1-Naphthylethylenediamine Dihydrochloride ( $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$ )
- Cadmium Powder (Technicon No. T11-5063)

\* Registered trademark of Atlas Chemical Industries, Inc.

- Hydrochloric Acid, concentrated (HCl)
- Copper Sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ )
- Potassium Nitrate ( $\text{KNO}_3$ )
- Chloroform ( $\text{CHCl}_3$ )
- Sulfuric Acid ( $\text{H}_2\text{SO}_4$ )
- Sodium Hydroxide (NaOH)
- Brij-35,\* 30% Solution (Technicon No. T21-0110)

### AMMONIUM CHLORIDE REAGENT

- Ammonium Chloride 35.5 ~~40~~ g
- Alkaline Water, q.s. 1000 ml
- Brij-35, 30% Solution 0.5 ml

Preparation: ~~10~~

EDTA 0.1 g.

Dissolve ~~10~~ g of ammonium chloride in alkaline water and dilute to one liter. Add 0.5 ml of Brij-35 per liter.

NOTE: Alkaline water is prepared by adding just enough ammonium hydroxide to distilled water to attain a pH of 8.5.

### COLOR REAGENT

- Sulfanilamide 20 g
- Concentrated Phosphoric Acid 200 ml
- N-1-Naphthylethylenediamine Dihydrochloride 1.0 g
- Distilled Water, q.s. 2000 ml
- Brij-35, 30% Solution 1.0 ml

Preparation:

To approximately 1500 ml of distilled water add 200 ml concentrated phosphoric acid and 20 g of sulfanilamide. Dissolve completely. (Heat if necessary.) Add 1.0 g of N-1-naphthylethylenediamine Dihydrochloride, and dissolve. Dilute to two liters. Add 1.0 ml of Brij-35. Store in a cold, dark place.

STABILITY: One month.

### CADMIUM, POWDER

Use coarse cadmium powder (99% pure). Rinse the powder once or twice with a little clean diethyl ether or 1N HCl followed by distilled water to remove grease and dirt. Allow the metal to air-dry and store in a well-stoppered bottle.

Bran + Luebbe, Inc. 1025 Busch Parkway Buffalo Grove, IL 60089-4516 • Phone (708) 520-0700 • Telefax (708) 520-0855



#### Preparation of Reductor Column:

The reductor column tube is a U-shaped fourteen-inch length of 2.0 mm I.D. glass tubing (Technicon No. 189-0000). Before filling the column, prepare the cadmium in the following manner:

- Cadmium metal is ground and sized. Particles are used in the column which pass a 25-mesh sieve but are held back by a 60-mesh sieve.
- New or used cadmium particles (10 grams) are cleaned with 50 ml of 6N HCl for 1 minute. Decant the HCl and wash the cadmium with another 50 ml of 6N HCl for 1 minute.
- Decant the HCl and wash the cadmium several times with distilled water.
- Decant the distilled water and add 50 ml of 2%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . Wash the cadmium until no blue color remains in solution.
- Rinse the cadmium several times with distilled water then decant.
- Add another 50 ml of 2%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and wash until no blue color remains in solution.
- Decant and wash thoroughly with distilled water.
- Fill the reductor column with Ammonium Chloride reagent and transfer the prepared cadmium particles to the column using a Pasteur pipette. Be careful not to allow any air bubbles to be trapped in the column. NOTE: In place of the Reductor Tube 189-0000, a 14-inch length of 0.081-in. I.D. Tygon tubing can be used.
- When the entire column is filled with granules, insert glass wool\*\* in both ends of the tube. Sleeve both ends with 0.090 I.D. Tygon tubing and insert an N-5 nipple on one side of the tube. Connect the other side of the tube directly to the A-2 debubbler by means of the 0.090 I.D. Tygon. Preparing the column in this fashion keeps it effective for hundreds of samples.
- Prior to sample analysis, condition the column with 100 mg N/l (nitrate) for 5 minutes followed by 100 mg N/l (nitrite) for 10 minutes.

#### STANDARDS

##### STOCK STANDARD A, 100 mg N/l

Potassium Nitrate	0.72	g
Distilled Water, q.s.	1000	ml
Chloroform	1	ml

##### Preparation:

Dissolve 0.72 g of potassium nitrate in distilled water and dilute to one liter. Store in a dark bottle. Add 1 ml of chloroform as a preservative.

##### STOCK STANDARD B, 20 mg N/l

Stock Standard A	20	ml
Distilled Water, q.s.	100	ml

##### Preparation:

Dilute 20 ml of Stock Standard A in a 100 ml volumetric flask with distilled water. Store in a dark bottle. Prepare fresh daily.

#### WORKING STANDARDS

ml Stock B	mg N/l
0.2	0.04
2.0	0.40
4.0	0.80
6.0	1.20
8.0	1.60
10.0	2.00

##### Preparation:

Pipette Stock B into a 100 ml volumetric flask. Dilute to 100 ml with distilled water. Store in a dark bottle. Prepare fresh daily.

#### OPERATING NOTES

- Do not preserve samples with  $\text{HgCl}_2$ .
- If samples must be kept beyond 24 hours, add two ml/l  $\text{H}_2\text{SO}_4$  ( $\text{pH} < 2$ ) and refrigerate.
- Samples containing residual chlorine should be pretreated before analyzing. (If treated as in [2], this is not necessary.)
- When running analyses on acid-preserved samples, be sure to adjust pH to between 5-9. (If ammonia is also to be run, adjust to between 5-7). To eliminate adjustment of samples individually, the ammonium chloride reagent can be made up to include 11.5 ml of 10% w/v NaOH per liter of solution.
- Samples containing grease or oil should be extracted with organic solvent first to prevent coating the cadmium. (Adjust 100 ml of sample to pH 2, extract with two 25-ml portions of Freon, or another suitable solvent; then readjust back to pH 6.)
- When samples contain a high concentration of metals, use ammonium chloride to which 0.1 g of disodium EDTA has been added. The  $\text{NH}_4\text{Cl}$  reagent must be adjusted to pH 8.5 before using the analytical system.
- When high concentrations of interference, such as metals, are present, or for mine-drainage samples, it is better to filter and dilute samples by 5:1 or 10:1 before assaying. (Range of method, if using AutoAnalyzer II system, can be lowered using STD CAL control at higher setting for these samples.)



8. Either centrifuge and decant or filter turbid samples.
9. It is of the utmost importance that the water used in preparing reagents and standards be completely free of contaminants.
10. In order to determine nitrate levels, the nitrite alone must be subtracted from the total (nitrate and nitrite). The nitrite value can be determined by eliminating the reductor column from the manifold, or by using the Technicon Method for Nitrite, Industrial Method No. 161-71W.
11. The reductor column must be clean and have good flow characteristics for the system to operate satisfactorily. Colloidal copper is the primary contaminant.
12. Never run distilled water wash while column is in system. Wash column with ammonium chloride, detach, close with tubing, and then wash out system.
13. To monitor column efficiency, run a standard after every 20 samples.
14. The pH of the ammonium chloride solution should always be checked to verify that it is 8.5. The use of 8.5 ammonium chloride, due to better buffering action, will normally prolong column life.
15. The efficiency of the reductor column has been found to be 99%. If the column has been properly prepared and properly treated, it

should be good for several hundred samples.

16. Before running the method, position the controls of the Modular Printer as follows:

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	40
RANGE Switch	200
DECIMAL Switch	0.00

Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

17. Alternate ranges may be obtained by utilization of the STD CAL control on the Colorimeter.
18. Sample cups should be washed with 1 N hydrochloric acid and rinsed thoroughly with deionized, distilled water in order to remove any traces of nitrate and nitrite.
19. The reagent baseline absorbance with reference to water should be approximately 0.06 absorbance units.
20. The Colorimeter should be operated in the Damp 1 mode.
21. The use of multiple working standards is only to establish linearity. For day-to-day operation, 1.2 mg N/l standard is recommended for instrument calibration.
22. For the most accurate results, only fresh Brij-35 should be used.

#### REFERENCES:

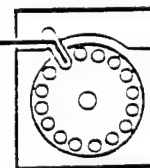
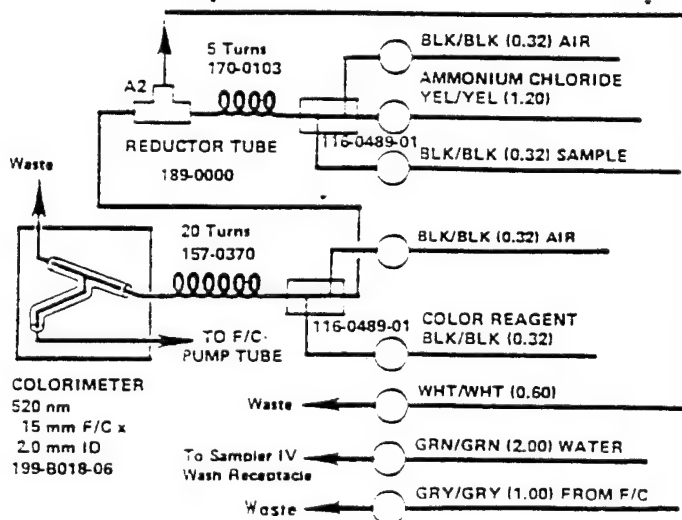
- 1 Armstrong, F.A.J., Sterns, C.R. and Strickland, J.D.H., 1967 Deep-Sea Res. 14, pp. 381-389. The measurement of upwelling and subsequent biological processes by means of the Technicon

AutoAnalyzer and associated equipment.

- 2 Grasshoff, K., Technicon International Congress, June, 1969.

- 3 Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, November, 1969.

#### NITRATE AND NITRITE IN WATER AND WASTEWATER (RANGE: 0.04-2.0 mg N/l) MANIFOLD No. 116-D049-01



SAMPLER/IV  
40 HR 4:1  
127/B128

NOTE: FIGURES IN PARENTHESES  
SIGNIFY FLOW RATES IN  
ML/MIN  
\*0.034 POLYETHYLENE



**Appendix B-8 – Lab Procedures for Ammonia Nitrogen:  
Method 350.1 (NH<sub>3</sub> Nitrogen)**



## Table of Contents – Appendix B-8

Documents
QC Document – Ammonia Nitrogen
Nitrogen, Ammonia Method 350.1 (Colorimetric, Automated Phenate)
LACHAT Instruments – Quik Chem Method 10-107-06-1-A
LACHAT Instruments – Quik Chem Method 10-107-06-1-B
Bran + Luebbe – AutoAnalyzer Application: Method 696D-82W



## Nitrogen, Ammonia - Method 350.1 (Colorimetric, Automated Phenate)

### 1.0 Procedure

Perform ammonia nitrogen (Method 350.1) analysis in accordance with procedures for the Technicon II AutoAnalyzer or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



# NITROGEN, AMMONIA

## Method 350.1 (Colorimetric, Automated Phenate)

STORET NO. Total 00610

Dissolved 00608

1. Scope and Application
  - 1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.01 to 2.0 mg/1  $\text{NH}_3$  as N. This range is for photometric measurements made at 630–660 nm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.
2. Summary of Method
  - 2.1 Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.
3. Sample Handling and Preservation
  - 3.1 Preservation by addition of 2 ml conc.  $\text{H}_2\text{SO}_4$  per liter and refrigeration at 4°C.
4. Interferences
  - 4.1 Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems during analysis. A 5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For sea water a sodium potassium tartrate solution is used.
  - 4.2 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.
5. Apparatus
  - 5.1 Technicon AutoAnalyzer Unit (AAI or AAI) consisting of:
    - 5.1.1 Sampler.
    - 5.1.2 Manifold (AAI) or Analytical Cartridge (AAII).
    - 5.1.3 Proportioning pump.
    - 5.1.4 Heating bath with double delay coil (AAI).
    - 5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 630–660 nm filters.
    - 5.1.6 Recorder.
    - 5.1.7 Digital printer for AAI (optional).

Approved for NPDES following preliminary distillation, Method 350.2.

Issued 1974

Editorial revision 1978



## 6. Reagents

- 6.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

**NOTE 1:** All solutions must be made using ammonia-free water.

- 6.2 Sulfuric acid 5N: Air scrubber solution. Carefully add 139 ml of conc. sulfuric acid to approximately 500 ml of ammonia-free distilled water. Cool to room temperature and dilute to 1 liter with ammonia-free distilled water.
- 6.3 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol in 500 ml of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with distilled water.
- 6.4 Sodium hypochlorite solution: Dilute 250 ml of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 ml with distilled water. Available chlorine level should approximate 2 to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.
- 6.5 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of distilled water.

**NOTE 2:** On salt water samples where EDTA solution does not prevent precipitation of cations, sodium potassium tartrate solution may be used to advantage. It is prepared as follows:

- 6.5.1 Sodium potassium tartrate solution: 10%  $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ . To 900 ml of distilled water add 100 g sodium potassium tartrate. Add 2 pellets of NaOH and a few boiling chips, boil gently for 45 minutes. Cover, cool, and dilute to 1 liter with ammonia-free distilled water. Adjust pH to  $5.2 \pm .05$  with  $\text{H}_2\text{SO}_4$ . After allowing to settle overnight in a cool place, filter to remove precipitate. Then add 1/2 ml Brij-35<sup>®</sup> (available from Technicon Corporation) solution and store in stoppered bottle.
- 6.6 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.
- 6.7 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride,  $\text{NH}_4\text{Cl}$ , dried at  $105^\circ\text{C}$ , in distilled water, and dilute to 1000 ml. 1.0 ml = 1.0 mg  $\text{NH}_3\text{-N}$ .
- 6.8 Standard Solution A: Dilute 10.0 ml of stock solution (6.7) to 1000 ml with distilled water. 1.0 ml = 0.01 mg  $\text{NH}_3\text{-N}$ .
- 6.9 Standard solution B: Dilute 10.0 ml of standard solution A (6.8) to 100.0 ml with distilled water. 1.0 ml = 0.001 mg  $\text{NH}_3\text{-N}$ .



- 6.10 Using standard solutions A and B, prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

<u>NH<sub>3</sub>-N, mg/l</u>	<u>ml Standard Solution/100 ml</u>
	<u>Solution B</u>
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0
	<u>Solution A</u>
0.20	2.0
0.50	5.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

**NOTE 3:** When saline water samples are analyzed, Substitute Ocean Water (SOW) should be used for preparing the above standards used for the calibration curve; otherwise, distilled water is used. If SOW is used, subtract its blank background response from the standards before preparing the standard curve.

<u>Substitute Ocean Water (SOW)</u>			
NaCl	24.53 g/l	NaHCO <sub>3</sub>	0.20 g/l
MgCl <sub>2</sub>	5.20 g/l	KBr	0.10 g/l
Na <sub>2</sub> SO <sub>4</sub>	4.09 g/l	H <sub>3</sub> BO <sub>3</sub>	0.03 g/l
CaCl <sub>2</sub>	1.16 g/l	SrCl <sub>2</sub>	0.03 g/l
KCl	0.70 g/l	NaF	0.003 g/l

## 7. Procedure

- 7.1 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples. For example, if the samples have been preserved with 2 ml conc. H<sub>2</sub>SO<sub>4</sub>/liter, the wash water and standards should also contain 2 ml conc. H<sub>2</sub>SO<sub>4</sub>/liter.
- 7.2 For a working range of 0.01 to 2.00 mg NH<sub>3</sub>-N/l (AAI), set up the manifold as shown in Figure 1. For a working range of .01 to 1.0 mg NH<sub>3</sub>-N/l (AAII), set up the manifold as shown in Figure 2. Higher concentrations may be accommodated by sample dilution.
- 7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through sample line.
- 7.4 For the AAI system, sample at rate of 20/hr, 1:1. For the AAII use a 60/hr 6:1 cam with a common wash.



- 7.5 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.
- 7.6 Switch sample line from distilled water to sampler and begin analysis.
8. Calculations
  - 8.1 Prepare appropriate standard curve derived from processing ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.
9. Precision and Accuracy
  - 9.1 In a single laboratory (EMSL), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg  $\text{NH}_3\text{-N/l}$ , the standard deviation was  $\pm 0.005$ .
  - 9.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.16 and 1.44 mg  $\text{NH}_3\text{-N/l}$ , recoveries were 107% and 99%, respectively.

### Bibliography

1. Hiller, A., and Van Slyke, D., "Determination of Ammonia in Blood", J. Biol. Chem. 102, p 499 (1933).
2. O'Connor, B., Dobbs, R., Villiers, B., and Dean, R., "Laboratory Distillation of Municipal Waste Effluents", JWPCF 39, R 25 (1967).
3. Fiore, J., and O'Brien, J. E., "Ammonia Determination by Automatic Analysis", Wastes Engineering 33, p 352 (1962).
4. A wetting agent recommended and supplied by the Technicon Corporation for use in AutoAnalyzers.
5. ASTM "Manual on Industrial Water and Industrial Waste Water", 2nd Ed., 1966 printing, p 418.
6. Booth, R. L., and Lobring, L. B., "Evaluation of the AutoAnalyzer II: A Progress Report" in Advances in Automated Analysis: 1972 Technicon International Congress, Vol. 8, p 7-10, Mediad Incorporated, Tarrytown, N.Y., (1973).
7. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 616. Method 604 (1975).



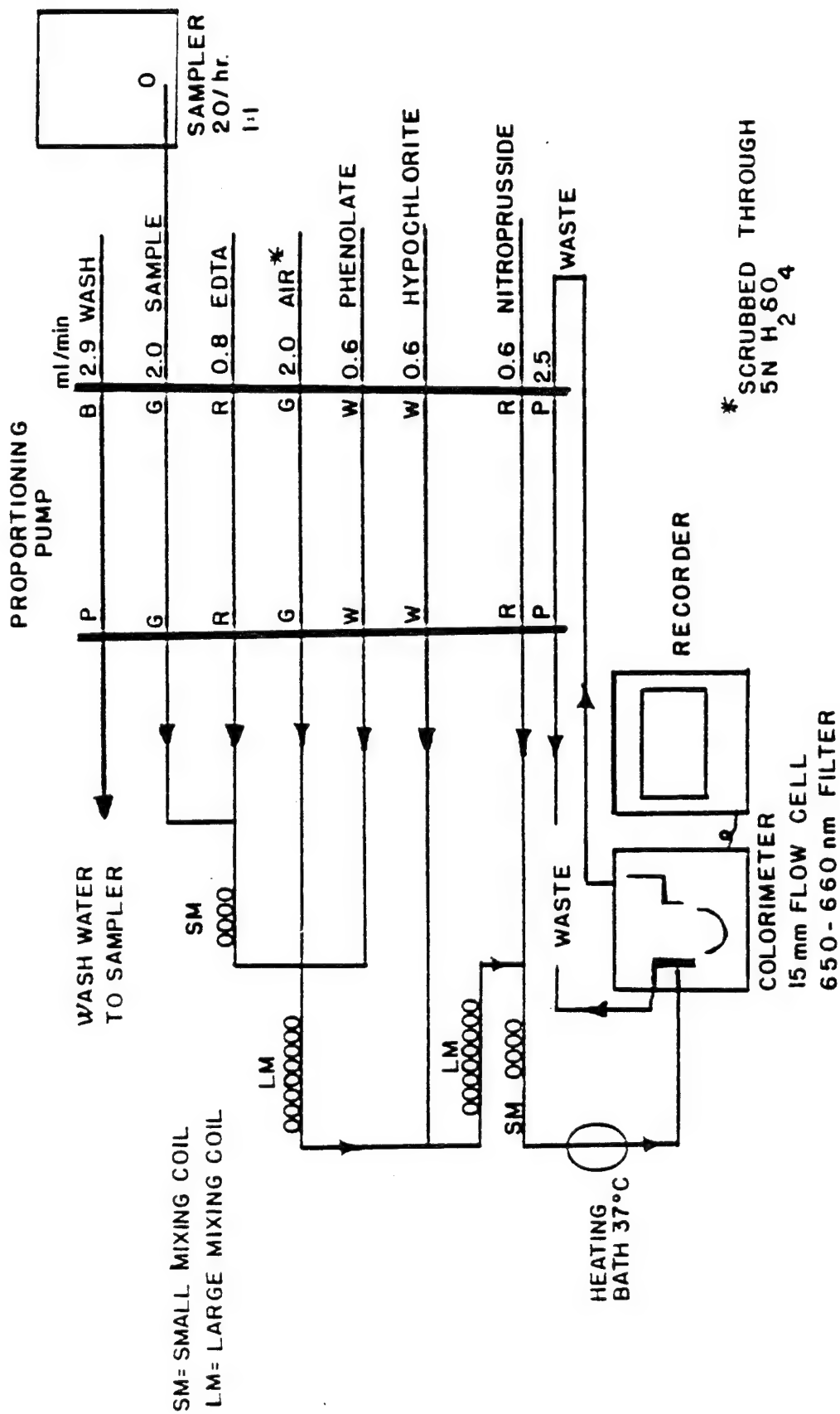


FIGURE 1 AMMONIA MANIFOLD AA I



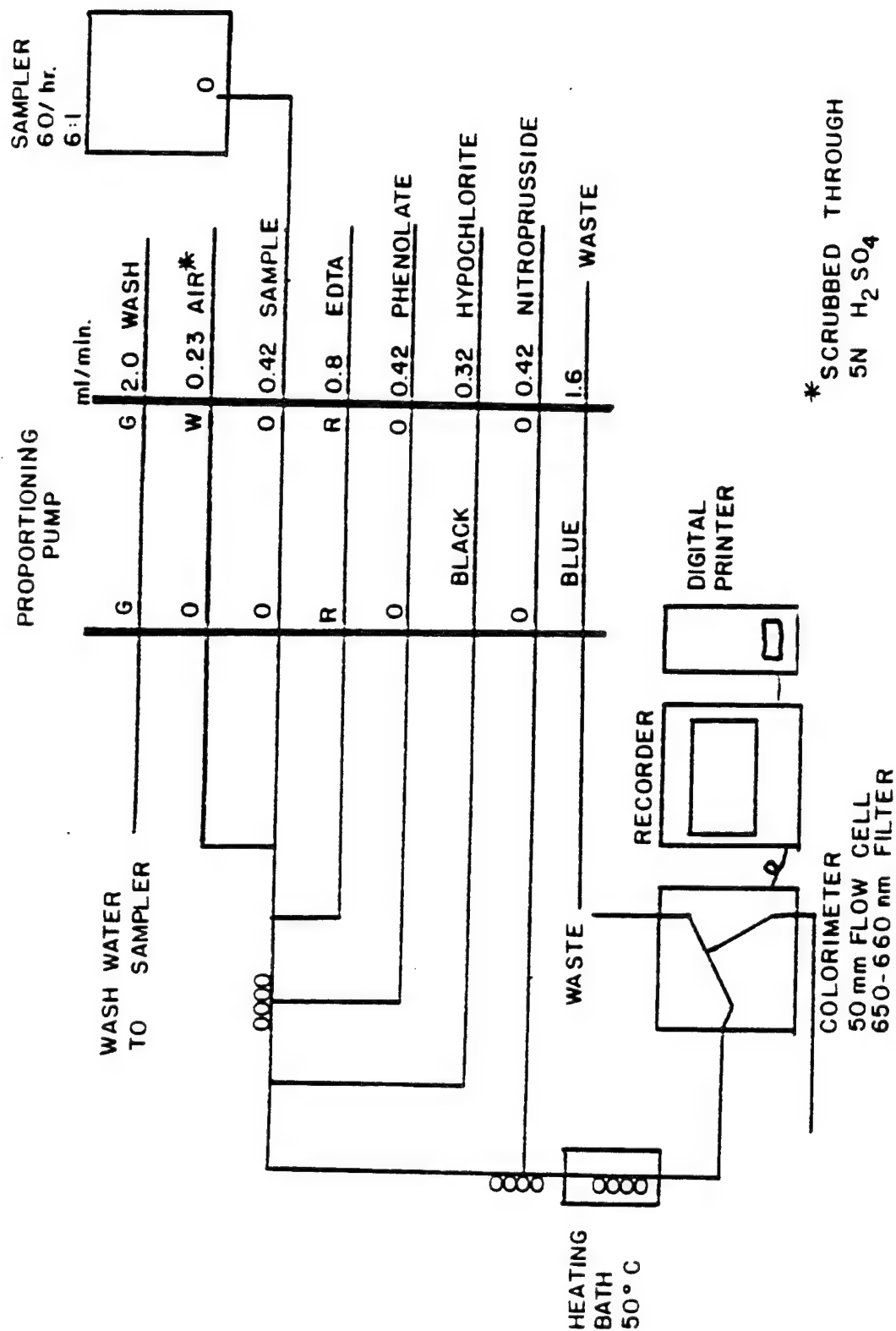


FIGURE 2. AMMONIA MANIFOLD AA II



QuikChem Method 10-107-06-1-A

## Ammonia (Phenolate) Potable and surface waters

0.1 to 20 mg N/L as  $\text{NH}_3$

### -- Principle --

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration.

### -- Interferences --

1. Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.
2. Color, turbidity and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation. See System Note 5 for specific instructions.

### -- Special Apparatus --

1. Heating Unit



## -- Sample Handling and Preservation --

Ammonia is volatile and will leave the sample slowly, even through polyethylene bottles. The samples should be run within 24 hours. If this cannot be done, they should be frozen. If this cannot be done, the samples should be adjusted to a pH of 3-5 with dilute phosphoric or sulfuric acid.

The Federal Register entry which defines standard EPA NPDES and NIPDWR methods states that "Manual distillation is NOT required if comparability data on representative effluent samples are on company file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies." (see SOURCES).

This suggests that the user perform recovery studies on representative sample types, studies which show that the undistilled samples give the same recoveries as the manually distilled samples. The manual distillation method referred to here, from EPA Method 350.2, is included here for your information.

### A. Distillation Reagents:

All reagents should be made up in distilled or deionized ammonia-free water.

**A-1. Sodium Hydroxide, 1 M:** Dissolve 40.0 g NaOH in 1.0 L water.

**A-2. Sodium Hydroxide, 0.1 M:** Dilute 1 M NaOH 1/10 by volume.

**A-3. Borate Buffer:** To a 1 L volumetric flask add 5.0 g anhydrous sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7$ ) or 9.5 g sodium borate decahydrate. Dissolve in about 500 mL water, then add 88 mL of 0.1 M NaOH and dilute to the mark. Invert three times.

**A-4. Boric Acid solution:** In a 1 L volumetric flask dissolve 20 g boric acid ( $\text{H}_3\text{BO}_3$ ) in distilled water and dilute to the mark. Invert three times.

**A-5. Nessler reagent:** In a beaker dissolve 100 g mercuric iodide ( $\text{HgI}_2$ ) and 70 g potassium iodide (KI) in a small amount of water. In a 1 L volumetric flask dissolve 160 g NaOH in 500 mL water and cool. Add the mixture in the beaker slowly, with stirring, to the cooled NaOH solution and dilute to the mark. Invert three times. If this reagent is stored out of direct sunlight it will remain stable for a period of up to 1 year.

### B. Distillation Procedure:

**Preparation of equipment:** Add 500 mL of distilled water to an 800 mL Kjeldahl flask. The addition of boiling chips which have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

**Sample Preparation:** Remove the residual chlorine in the sample by adding dechlorinating agent equivalent to the chlorine residual. To 400 mL of sample add 1 M NaOH (A-1. above) until the pH is 9.5, checking the pH during addition with a pH meter or by use of a short range pH paper.



**Distillation:** Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 mL Kjeldahl flask and add 25 mL of the borate buffer (A-3. above). Distill 300 mL at the rate of 6-10 mL/min. into 50 mL of 2% boric acid (A-4. above) contained in a 500 mL Erlenmeyer flask.

**NOTE:** The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

Dilute the distillate to 500 mL with distilled water.

### -- Preparation of Reagents --

Use deionized water (10 megohm) for all solutions.

#### Degassing with helium

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140 kPa (20 lb/in<sup>2</sup>) through a helium degassing tube (Lachat Part 50100). Bubble He vigorously through the solution for one minute.

#### Reagent 1. Sodium Phenolate

**CAUTION:** Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.

**By Volume:** In a 1 L volumetric flask, dissolve **88 mL of 88% liquified phenol** or **83 g crystalline phenol** (C<sub>6</sub>H<sub>5</sub>OH) in approximately **600 mL water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and invert three times. Do not degas this reagent.

**By Weight:** To a tared 1 L container, add **888 g water**. Add **94.2 g of 88% liquified phenol** or **83 g crystalline phenol** (C<sub>6</sub>H<sub>5</sub>OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and invert three times to mix thoroughly. Do not degas this reagent.

#### Reagent 2. Sodium Hypochlorite

**By Volume:** In a **500 mL** volumetric flask, dilute **250 mL Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] to mark with **water**. Invert three times to mix.

**By Weight:** To a **500 mL** tared container add **250 g Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] and **250 g water**. Stir or shake to mix.



**Reagent 3. Buffer**

**By Volume:** In a 1 L volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetate** ( $\text{Na}_2\text{EDTA}$ ) and **5.5 g sodium hydroxide** ( $\text{NaOH}$ ) in about **900 mL water**. Dilute to the mark and invert three times.

**By Weight:** To a tared 1 L container add **50.0 g disodium ethylenediamine tetraacetate** ( $\text{Na}_2\text{EDTA}$ ) and **5.5 g sodium hydroxide** ( $\text{NaOH}$ ). Add **968 g water**. Shake several times.

**Reagent 4. Sodium Nitroprusside**

**By Volume:** In a 1 L volumetric flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferricyanide [ $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$ ]) dilute to the mark with **water**.

**By Weight:** To a tared, 1 L flask, add **3.50 g sodium nitroprusside** (Sodium Nitroferricyanide [ $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$ ]) and **1000 g water**. Stir or shake to mix.

**-- Preparation of Standards --****Standard 1. Stock Standard 10000.0 mg N/L as  $\text{NH}_3$** 

In a 1 L volumetric flask dissolve **38.19 g ammonium chloride** ( $\text{NH}_4\text{Cl}$ ) that has been dried for two hours at  $110^\circ\text{C}$  in about **800 mL water**. Dilute to the mark and invert three times.

**Standard 2. Intermediate Stock Standard 200.0 mg N/L as  $\text{NH}_3$** 

**By Volume:** To a 1 L volumetric flask, add **20.0 mL Stock Standard** (Standard 1) and dilute to the mark with **water**. Invert three times.

**By Weight:** To a tared 1 L container add about **20 g Stock Standard** (Standard 1). Measure the exact weight of the solution added and divide this weight by **0.02**. This will give you the total weight of the diluted solution to be made. Make up the solution to this weight with **water**. Shake before using.

**Set of Seven Working Standards:**

**20.0, 8.00, 2.00, 1.00, 0.400, 0.200, and 0.100 mg N/L**

**By Volume:** To seven **250 mL** volumetric flasks add, respectively, exactly

**25.0 10.0 2.50 1.250 0.500 0.250 0.125 mL**

of the **Intermediate Stock Standard** (Standard 2). Dilute each to the mark with **water** and invert three times.



**By Weight:** To seven tared **250 mL** containers add, respectively, about

**25    10    2.5    1.25    0.5    0.25    0.125    g**

of the **Intermediate Stock Standard** (Standard 2). Take the exact weight obtained for each and divide by

**0.1    0.04    0.01    0.005    0.002    0.001    0.0005**

to get the total weight of the diluted solution. Make up each solution to this total weight using a wash bottle filled with **water**. Shake before using.

As the analyte is volatile, especially at low concentrations, the standards should be used within two days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

### -- Timing --

Sample throughput: 90 samples/h: 40 s/sample  
Pump speed: 35

Cycle period: 40 s  
Inject to start of peak period: 25 s  
Inject to end of peak period: 63 s

### -- System Notes --

1. Allow 15 min for heating unit to warm up to 60°C.
2. System IV GAIN: 175 x 1. AE instrument, top scale response = 1.0 abs.
3. If standards are not distilled samples should be multiplied by 500/400 to correct for the increase in volume after the distillation.
4. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:
  1. Place all reagent lines in deionized water and pump to clear reagents (2-5 minutes).
  2. Place reagent lines and carrier in 1 M hydrochloric acid (1 volume conc HCl added to 11 volumes of deionized water) and pump for several minutes.
  3. Place all lines in deionized water and pump until the HCl is thoroughly washed out.
  4. Resume pumping reagents



5. If samples are colored or are suspected to show a background absorbance, this interference should be subtracted. This can be done by the following procedure:
  - a. Calibrate the system in the normal manner. (If using a 4000 series instrument, the baseline subtraction feature must be used.)
  - b. Disable the check standard or DQM features and analyze the samples.
  - c. Place reagent and carrier lines in DI water and allow the baseline to stabilize.
  - d. Inject samples again without recalibrating.
  - e. Subtract the "background" concentration from the original concentration to give the corrected concentration.

$$\text{Corrected Concentration} = \text{Original Concentration} - \text{Background Concentration}$$

#### -- Waste Mangement --

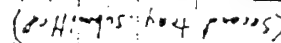
Laboratory waste practices should be conducted consistent with all applicable rules and regulations. For further information on waste management, consult **The Waste Management Manual for Laboratory Personnel** and **Less is Better: Laboratory Chemical Management for Waste Reduction**, available from the American Chemical Society's Department of Regulations and Science Policy, 1155 16th Street N.W. Washington, D.C., U.S.A. 20036. (202) 872-4477.

#### -- Sources --

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1



11





**QuikChem METHOD 10-107-06-1-B**

**DETERMINATION OF AMMONIA (PHENOLATE)  
BY FLOW INJECTION ANALYSIS COLORIMETRY**

Written by William R. Prokopy

Applications Group

Revision date:

24 December 1993

LACHAT INSTRUMENTS

6645 WEST MILL ROAD

MILWAUKEE, WI 53218, USA



# LACHAT

INSTRUMENTS

QuikChem Method 10-107-06-1-B

## Ammonia (Phenolate) in Potable and Surface Waters

0.01 to 2.0 mg N/L

### -- Principle --

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

### -- Interferences --

1. Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.
2. Color, turbidity and certain organic species may interfere. Turbidity is removed by manual filtration.

### -- Special Apparatus --

1. Heating Unit.



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## QUIKCHEM METHOD 10-107-06-1-B

# DETERMINATION OF AMMONIA BY FLOW INJECTION ANALYSIS COLORIMETRY

### 1. SCOPE AND APPLICATION

- 1.1. This method covers the determination of ammonia in potable and surface waters.
- 1.2. The method is based on reactions that are specific for ammonia.
- 1.3. The applicable range is 0.01 to 2.0 mg N/L. The method detection limit is 0.006 mg N/L. Approximately 70 samples per hour can be analyzed.

### 2. SUMMARY OF METHOD

- 2.1. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

### 3. DEFINITIONS

- 3.1. CALIBRATION BLANK (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.
- 3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.4. LABORATORY FORTIFIED BLANK (LFB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.5. LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background



concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

- 3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.
- 3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.
- 3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

#### 4. INTERFERENCES

- 4.1. Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.
- 4.2. Color, turbidity and certain organic species may interfere. Turbidity is removed by manual filtration.

#### 5. SAFETY

- 5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.
- 5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all



personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Phenolate

5.3.2. Nitroferricyanide

## **6. EQUIPMENT AND SUPPLIES**

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Autosampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

## **7. REAGENTS AND STANDARDS**

### **7.1 PREPARATION OF REAGENTS**

Use deionized water (10 megohm) for all solutions.

#### **Degassing with helium:**

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140 kPa (20 lb/in<sup>2</sup>) through a helium degassing tube (Lachat Part 50100). Bubble He vigorously through the solution for one minute.



### Reagent 1. Sodium Phenolate

**CAUTION:** Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.

**By Volume:** In a 1 L volumetric flask dissolve 88 mL of 88% liquified phenol or 83 g crystalline phenol ( $C_6H_5OH$ ) in approximately 600 mL water. While stirring, slowly add 32 g sodium hydroxide (NaOH). Cool, dilute to the mark, and invert three times. Do not degas this reagent.

**By Weight:** To a tared 1 L container, add 888 g water. Add 94.2 g of 88% liquified phenol or 83 g crystalline phenol ( $C_6H_5OH$ ). While stirring, slowly add 32 g sodium hydroxide (NaOH). Cool and invert three times to mix thoroughly. Do not degas this reagent.

### Reagent 2. Sodium Hypochlorite

**By Volume:** In a 500 mL volumetric flask dilute 250 mL Regular Clorox bleach [5.25% sodium hypochlorite ( $NaOCl$ ), The Clorox Company, Oakland, CA] to the mark with water. Invert to mix.

**By Weight:** To a tared 500 mL container add 250 g Regular Clorox bleach [5.25% sodium hypochlorite ( $NaOCl$ ), The Clorox Company, Oakland, CA] and 250 g water. Stir or shake to mix.

### Reagent 3. Buffer

**By Volume:** In a 1 L volumetric flask, dissolve 50.0 g disodium ethylenediamine tetraacetate ( $Na_2EDTA$ ) and 5.5 g sodium hydroxide (NaOH) in approximately 900 mL water. Dilute to the mark and mix with a magnetic stirrer until dissolved.

**By Weight:** To a 1 L tared container add 50.0 g disodium ethylenediamine tetraacetate ( $Na_2EDTA$ ) and 5.5 g sodium hydroxide (NaOH). Add 968 g water. Mix with a magnetic stirrer until dissolved.

### Reagent 4. Sodium Nitroprusside

**By Volume:** To a 1 L volumetric flask dissolve 3.50 g sodium nitroprusside (Sodium Nitroferricyanide [ $Na_2Fe(CN)_5NO \cdot 2H_2O$ ]) dilute to the mark with water and invert to mix.

**By Weight:** To a tared 1 L container add 3.50 g sodium nitroprusside (Sodium Nitroferricyanide [ $Na_2Fe(CN)_5NO \cdot 2H_2O$ ]) and 1000 g water. Invert to mix.



## 7.2 PREPARATION OF STANDARDS

### Standard 1. Stock Standard 1000 mg N/L

In a 1 L volumetric flask dissolve 3.819 g ammonium chloride ( $\text{NH}_4\text{Cl}$ ) that has been dried for two hours at  $110^\circ\text{C}$  in about 800 mL water. Dilute to the mark with DI water and invert to mix.

### Standard 2. Working Stock Standard Solution 20.0 mg N/L

**By Volume:** In a 1 L volumetric flask, add 20.0 mL Stock Standard (Standard 1) to the mark with water. Invert to mix.

**By Weight:** To a tared 1 L container add about 20 g Stock Standard (Standard 1). Divide the actual weight of the solution added by 0.02 and make up to this resulting total weight with water. Invert to mix.

### Set of Eight Working Standards:

2.00	0.80	0.20	0.100	0.040	0.020	0.010	0.00	mg N/L
------	------	------	-------	-------	-------	-------	------	--------

**By Volume:** To eight 250 mL volumetric flasks add, respectively,

25.0	10.0	2.50	1.25	0.50	0.250	0.125	0.0	mL
------	------	------	------	------	-------	-------	-----	----

Standard 2. Dilute each to the mark with water invert to mix.

**By Weight:** To eight tared 250 mL containers add, respectively, about

25.0	10.0	2.50	1.25	0.50	0.250	0.125	0.0	g
------	------	------	------	------	-------	-------	-----	---

Standard 2. Take the exact weight obtained for each and divide by

0.10	0.04	0.01	0.005	0.002	0.001	0.0005	
------	------	------	-------	-------	-------	--------	--

to get the total weight of the diluted solution. Make up each solution to this total weight with water. Shake before using.

If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

## 8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water volume collected should be sufficient to insure a



representative sample, allow for replicate analysis (if required), and minimize waste disposal.

- 8.2. Ammonia is volatile and will leave the sample slowly, even through polyethylene bottles. The samples should be run within 24 hours. If this cannot be done, they should be frozen. If this cannot be done, the samples should be adjusted to a pH of 3-5 with dilute phosphoric or sulfuric acid. The Federal Register entry which defines standard EPA NPDES and NPDWR methods states that "Manual Distillation is NOT required if comparability data on representative effluent samples are on company file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies". (see SOURCES). This suggests that the user perform recovery studies on representative sample types, studies which show that the undistilled samples give the same recoveries as the manually distilled samples. Follow EPA Method 350.2 for the complete distillation procedure.

## 9. QUALITY CONTROL (USEPA GUIDELINE)

- 9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

### 9.2. INITIAL DEMONSTRATION OF PERFORMANCE

- 9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.
- 9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by  $\pm 10\%$ , linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.
- 9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within  $\pm 10\%$  of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with ongoing analyses.



9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates]. S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

### 9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery ( $\bar{X}$ ) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$UPPER CONTROL LIMIT = \bar{X} + 3S$$

$$LOWER CONTROL LIMIT = \bar{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.



9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

#### 9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

Where, R = percent recovery

$C_s$  = fortified sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.



## 10. CALIBRATION AND STANDARDIZATION

- 10.1. Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).
- 10.4. Calibration the instrument as description in section 11.
- 10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.
- 10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed  $\pm 10\%$  of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

## 11. PROCEDURE

### 11.1. Calibration Procedure

- 11.2.1. Prepare reagent and standards as described in section 7.
- 11.2.2. Set up manifold as shown in section 17.1.
- 11.2.3. Input peak timing and integration window parameters as below:

Sample throughput: 72 samples/h; 50 s/sample

Pump speed: 35

Cycle period: 72 s

Inject to start of peak period: 22 s

Inject to end of peak period: 68 s

Presentation, Data Window

Top Scale Response: 0.50 abs

Bottom Scale Response: 0.00 abs

Segment/Boundaries: A: 2.00 mg N/L

D: 0.10 mg N/L



G: 0.01 mg N/L

H: 0.00 mg N/L

Series 4000/System IV Settings: Gain = 500 x 1

- 11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.
- 11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

### 11.3. Analysis Procedure

- 11.3.1. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).

### 11.4. System Notes

- 11.4.1. Allow 15 min for heating unit to warm up to 60°C.
- 11.4.2. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure.
  - A. Place all reagent lines in deionized water and pump to clear reagents (2 to 5 min).
  - B. Place all reagent lines in 1 M hydrochloric acid (1 volume concentrated HCl added to 11 volumes of deionized water) and pump for several minutes.
  - C. Place all reagent lines in deionized water and pump until the HCl is thoroughly washed out.
  - D. Resume pumping reagents.

## 12. DATA ANALYSIS AND CALCULATIONS

- 12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve.



- 12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

12.3. Report results in mg N/L.

### **13. METHOD PERFORMANCE**

- 13.1. The method performance data are presented as method support data in section 17.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

### **14. POLLUTION PREVENTION**

- 14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.
- 14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy, 115 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

### **15. WASTE MANAGEMENT**

- 15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.



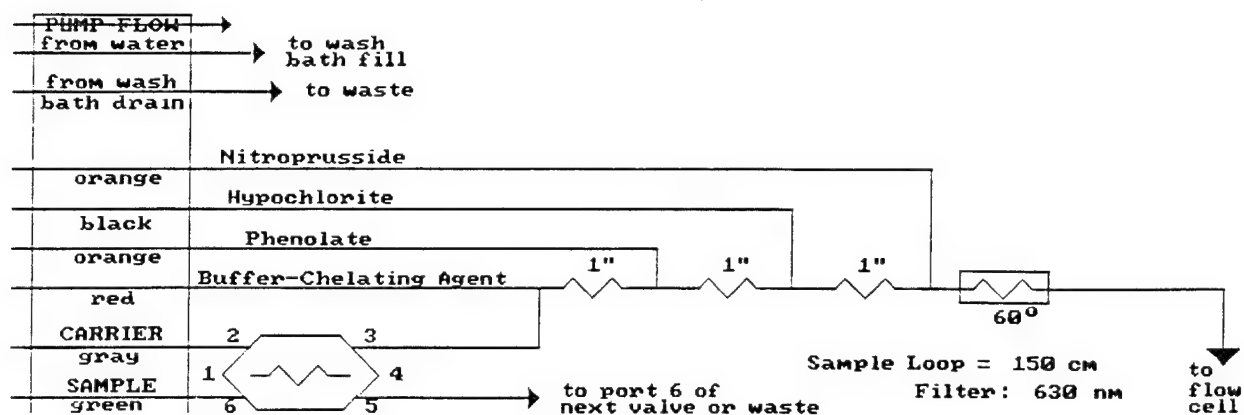
## 16. REFERENCES

- 16.1. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 350.1



## 17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

### 17.1. MANIFOLD DIAGRAM:



**CARRIER** is Water.

1" is 70.0 cm of tubing on a 1 in coil support

2" is 135 cm of tubing on a 2 in. coil support

**APPARATUS:** Standard valve, flow cell, and detector head modules are used. The box shows 650 cm of heated tubing.

All manifold tubing is 0.8 mm (0.032 in.) i.d. This is 5.2 uL/cm.

**MANIFOLD DIAGRAM REVISION DATE:** 12 August 1993 by N. Liao.



## 17.2. SUPPORT DATA:

### 17.2.1. Support Data

QuikChem AE Calibration Report for Calibration 93121001

Method: ammonia 10-107-06-1-B

This calibration was first done or last verified on 12/10/93 at 12:13 pm

This report prepared on 12/10/93 at 01:18 pm

Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard A, ammonia	mg N/L	2.000	1.991	0.46	0.1157
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard B, ammonia	mg N/L	0.800	0.822	-2.77	0.0488
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard C, ammonia	mg N/L	0.200	0.207	-3.65	0.0123
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard D, ammonia	mg N/L	0.100	0.096	3.71	0.0059
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard E, ammonia	mg N/L	0.040	0.040	-0.65	0.0026
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard F, ammonia	mg N/L	0.020	0.014	30.01	0.0011
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard G, ammonia	mg N/L	0.010	0.010	0.00	0.0006
Analyte	Units	Average Concentrations			Baseline Corrected Average Absorbance
		Known	Determined	% Residual	
Standard H, ammonia	mg N/L	0.000	0.000	***.00	-0.0000

Cal Ref: 93121001  
Method: AMMONB

#### Calibration Statistics Report

12/10/93 01:21 pm

Channel: ammonia

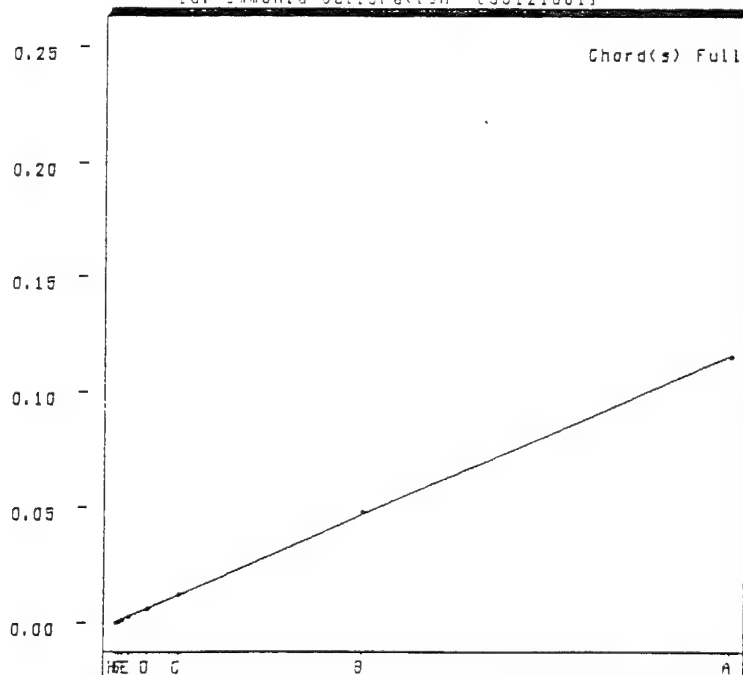
#### Correlation Coefficients

Seg	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-G	0.9998	0.9772 ?	0.9998	0.9997	0.9998	0.9994
2	G-H	0.9910	0.7363 ?	0.9787 ?	0.9953	0.9911	0.2604 ?

#### Percent Standard Deviation in Slope

1	A-G	0.6	6.2	0.5	0.8	0.5	1.0
2	G-H	6.7	42.3	10.4	4.8	6.7	119.2 ?

1a. ammonia Calibration (93121001)



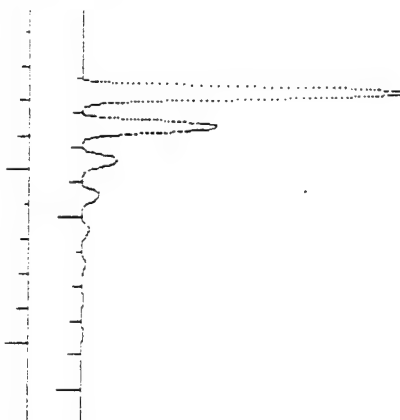
Std	mg N/L
A	2.0000
B	0.8000
C	0.2000
D	0.1000
E	0.0400
F	0.0200
G	0.0100
H	0.0000

Calibration Sequence: ABCDEFGHH



Calibration 12/10/1993, 12:01 pm

1. Standard A (1)
2. Standard B (1)
3. Standard C (1)
4. Standard D (1)
5. Standard E (1)
6. Standard F (1)
7. Standard G (1)
7. Standard G (2)
8. Standard H (1)
8. Standard H (2)



Rack 1 (Ref: 93121001) 12/10/1993, 12:17 pm

101. 0.01 ug N/L (1)
102. 0.01 ug N/L (1)
103. 0.01 ug N/L (1)
104. 0.01 ug N/L (1)
105. 0.01 ug N/L (1)
106. 0.01 ug N/L (1)
107. 0.01 ug N/L (1)
108. 0.01 ug N/L (1)
109. 0.01 ug N/L (1)
110. 0.01 ug N/L (1)
111. 0.01 ug N/L (1)
112. 0.01 ug N/L (1)
113. 0.01 ug N/L (1)
114. 0.01 ug N/L (1)
115. 0.01 ug N/L (1)
116. 0.01 ug N/L (1)
117. 0.01 ug N/L (1)
118. 0.01 ug N/L (1)
119. 0.01 ug N/L (1)
120. 0.01 ug N/L (1)
121. 0.01 ug N/L (1)

Cash Sample ID

- 101 0.01 ug N/L
- 102 0.01 ug N/L
- 103 0.01 ug N/L
- 104 0.01 ug N/L
- 105 0.01 ug N/L
- 106 0.01 ug N/L
- 107 0.01 ug N/L
- 108 0.01 ug N/L
- 109 0.01 ug N/L
- 110 0.01 ug N/L
- 111 0.01 ug N/L
- 112 0.01 ug N/L
- 113 0.01 ug N/L
- 114 0.01 ug N/L
- 115 0.01 ug N/L
- 116 0.01 ug N/L
- 117 0.01 ug N/L
- 118 0.01 ug N/L
- 119 0.01 ug N/L
- 120 0.01 ug N/L
- 121 0.01 ug N/L

assay  
ug N/L

- 0.010
- 0.009
- 0.009
- 0.009
- 0.012
- 0.011
- 0.012
- 0.011
- 0.010
- 0.010
- 0.011
- 0.011
- 0.010
- 0.011
- 0.010
- 0.008
- 0.011
- 0.012
- 0.013
- 0.012
- 0.009

MDL

$\bar{x} = 0.011$  mg N/L

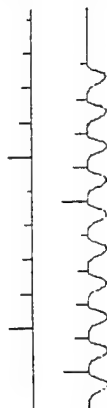
$s = 0.0013$

MDL = 0.003 mg N/L



Rack 1 (Ref: 93121002) 12/10/1993, 12:48 pm

101. 0.1 ug N/L (1)  
102. 0.1 ug N/L (1)  
103. 0.1 ug N/L (1)  
104. 0.1 ug N/L (1)  
105. 0.1 ug N/L (1)  
106. 0.1 ug N/L (1)  
107. 0.1 ug N/L (1)  
108. 0.1 ug N/L (1)  
109. 0.1 ug N/L (1)  
110. 0.1 ug N/L (1)



Cup# Sample ID

101 0.1 ug N/L  
102 0.1 ug N/L  
103 0.1 ug N/L  
104 0.1 ug N/L  
105 0.1 ug N/L  
106 0.1 ug N/L  
107 0.1 ug N/L  
108 0.1 ug N/L  
109 0.1 ug N/L  
110 0.1 ug N/L

1  
assonia  
ug N/L

Precision

x = 0.105 mg N/L

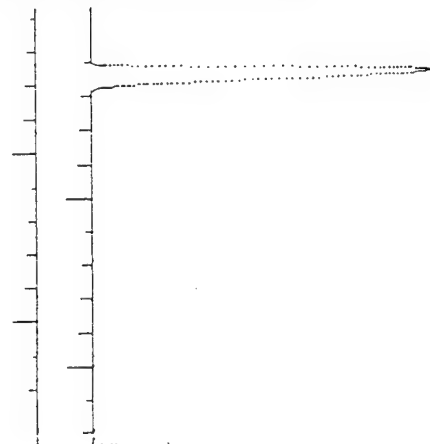
s = 0.002

RSD = 1.90%

0.104  
0.106  
0.109  
0.106  
0.103  
0.102  
0.105  
0.106  
0.107  
0.105

Rack 1 (Ref: 93121003) 12/10/1993, 01:01 pm

101. 2 ug N/L (1)  
102. blank (1)  
103. blank (1)  
104. blank (1)  
105. blank (1)  
106. blank (1)  
107. blank (1)  
108. blank (1)  
109. blank (1)  
110. blank (1)  
111. blank (1)  
112. blank (1)



Cup# Sample ID

101 2 ug N/L  
102 blank  
103 blank  
104 blank  
105 blank  
106 blank  
107 blank  
108 blank  
109 blank  
110 blank  
111 blank  
112 blank

assonia  
ug N/L

Carryover

x = -0.0004 mg N/L

s = 0.0012

t = 2.262

-0.0004 +/- 0.0009

EMDL

s = 0.0012

EMDL = 0.006 mg N/L

2.072  
-0.002  
0.000  
-0.001  
-0.001  
0.001  
0.000  
0.001  
-0.000  
0.000  
-0.003  
-0.001



---

## *AutoAnalyzer Application*

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Method - 696D-82W

### **AMMONIA IN WATER AND WASTEWATER**

Range: 0.02-3 mg/l N

#### **Description**

The automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of EDTA is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.<sup>1,2,3,4,5,6</sup> Sodium nitroprusside is added to intensify the blue color.

#### **Reagents**

Unless otherwise specified, all chemicals should be of ACS grade or equivalent.

#### **List of Raw Materials**

Ammonium Sulfate ( $\text{NH}_3)_2\text{SO}_4$ ) Primary Standard Grade  
Brij-35, 30% Solution\*  
Disodium, ethylenediamine-tetracetate  
Phenol Liquified, approx. 90% ( $\text{C}_6\text{H}_5\text{OH}$ )  
Sodium Hydroxide, 50 % w/w Solution ( $\text{NaOH}$ )  
Sodium Hypochlorite, 5% Solution ( $\text{NaOCl}$ ) Commercial Grade; e.g. Clorox  
Sodium Nitroprusside ( $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ )  
Sulfuric Acid, conc. ( $\text{H}_2\text{SO}_4$ )

\* \* \* \* \*

\* Trademark of Atlas Chemical

1. Van Slyke, D.D. and Hillern, A.J., BioChem., 102, p.499, 1933
2. Kallman, S., Presentation at Div. 1 Meeting of ASTM Committee E-3, April, 1967, San Diego, CA.
3. Bolleter, W.T., Bushman, C.J. and Tidwell, P.N., Anal. Chem., 33, P.592, 1961.
4. Tellow, J.A. and Wilson, A.L., Analyst, 89, p.453, 1964.
5. Tarugi, A. and Lenci, F., Boll Chim. Farm., 50, p.9070, 1912.
6. WPCA Methods of Chem. Anal. of Water & Wastewater, November 1969, p.137.



# BRAN+LUEBBE

THE POWER OF PRECISION

## Method No. 696D-82W (cont'd)

Multi-Test

Page 2

### Reagent Make-Up

All water used in reagent and standard preparation must be ammonia free.

#### Alkaline Phenol

Phenol, Liquified

70 g 62 ml

Sodium Hydroxide Solution, 50% w/w

53.4 g 36 ml

Distilled Water, qs

1000 ml

#### Preparation:

Add 70 g of liquified phenol to about 800 ml of distilled water. While cooling under tap water or in an ice bath, slowly with swirling add 53.4 g of sodium hydroxide solution, 50% w/w/. Cool to room temperature, dilute to 1000 ml with distilled water and mix thoroughly. Store in an amber glass container. This material is corrosive.

Stability: Two weeks.

#### Sodium Hypochlorite Solution

Sodium Hypochlorite Solution, 5%

210 ml

Distilled Water, qs

500 ml

#### Preparation:

Dilute 210 ml of sodium hypochlorite solution 5% to 500 ml with distilled water and mix thoroughly. Prepare fresh weekly.

#### Sodium Nitroprusside Solution

Sodium Nitroprusside

3.5 g

Distilled Water, qs

1000 ml

#### Preparation:

Dissolve 3.5 g of sodium nitroprusside in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly. Store in an amber container.

Stability: One month.

#### Disodium EDTA

Disodium EDTA

33.6 g

Sodium Hydroxide, 50% w/w

0.8 g

Distilled Water, qs

1000 ml

Brij-35

2 ml

#### Preparation:

Dissolve approximately 0.8 g of 50% w/w sodium hydroxide and 33.6 g of disodium EDTA in about 800 ml of distilled water. Dilute to one liter. Add 2 ml of Brij-35 and mix well.



# BRAN+LUEBBE

THE POWER OF PRECISION

## Method No. 696D-82W (cont'd)

Multi-Test

Page 3

### Standard Preparation

#### Stock standard A, 100 mg/l N

Ammonium Sulfate

0.4717 g

Chloroform

1 ml

Distilled Water, qs

1000 ml

#### Preparation:

In a one liter volumetric flask containing about 800 ml of distilled water dissolve 1 ml of chloroform. Add 0.4717 g of ammonium sulfate and swirl to dissolve. Dilute to one liter with distilled water and mix thoroughly.

#### Stock Standard B, 20 mg/l N

Stock Standard A

20.0 ml

Distilled Water, qs

100 ml

#### Preparation:

Dilute 20.0 ml of stock standard A to 100 ml with distilled water and mix thoroughly. Prepare fresh daily.

### Working Standard Solutions

<u>ml Stock B</u>	<u>mg/l N</u>
2.0	0.4
4.0	0.8
6.0	1.2
8.0	1.6
10.0	2.0
15.0	3.0

#### Preparation:

Transfer aliquots of Stock Standard B as noted above, to individual 100 ml volumetric flasks. Dilute to volume with distilled water and mix thoroughly. Prepare fresh daily.



# BRAN+LUEBBE

THE POWER OF PRECISION

Method No. 696D-82W (cont'd)

Multi-Test

Page 4

## Sample Preparation

1. Preserve by addition of 2 ml/l  $H_2SO_4$  and refrigerate at 4°C.
2. Sample turbidity should be removed by filtration prior to analysis. It may interfere with this chemistry.
3. Sample cups can be rinsed with 1:1 hydrochloric acid, followed by distilled water and finally by an aliquot of the sample itself.

## System Cleansing Procedure

Pump 5N  $H_2SO_4$  as the cleaning solution.

## System Performance

Maximum reagent/water baseline

Reagent Equilibration time

Sensitivity of high standard at a STD CAL of 1.0

Coefficient of Variation

0.040 AU

5 min

0.22 AU

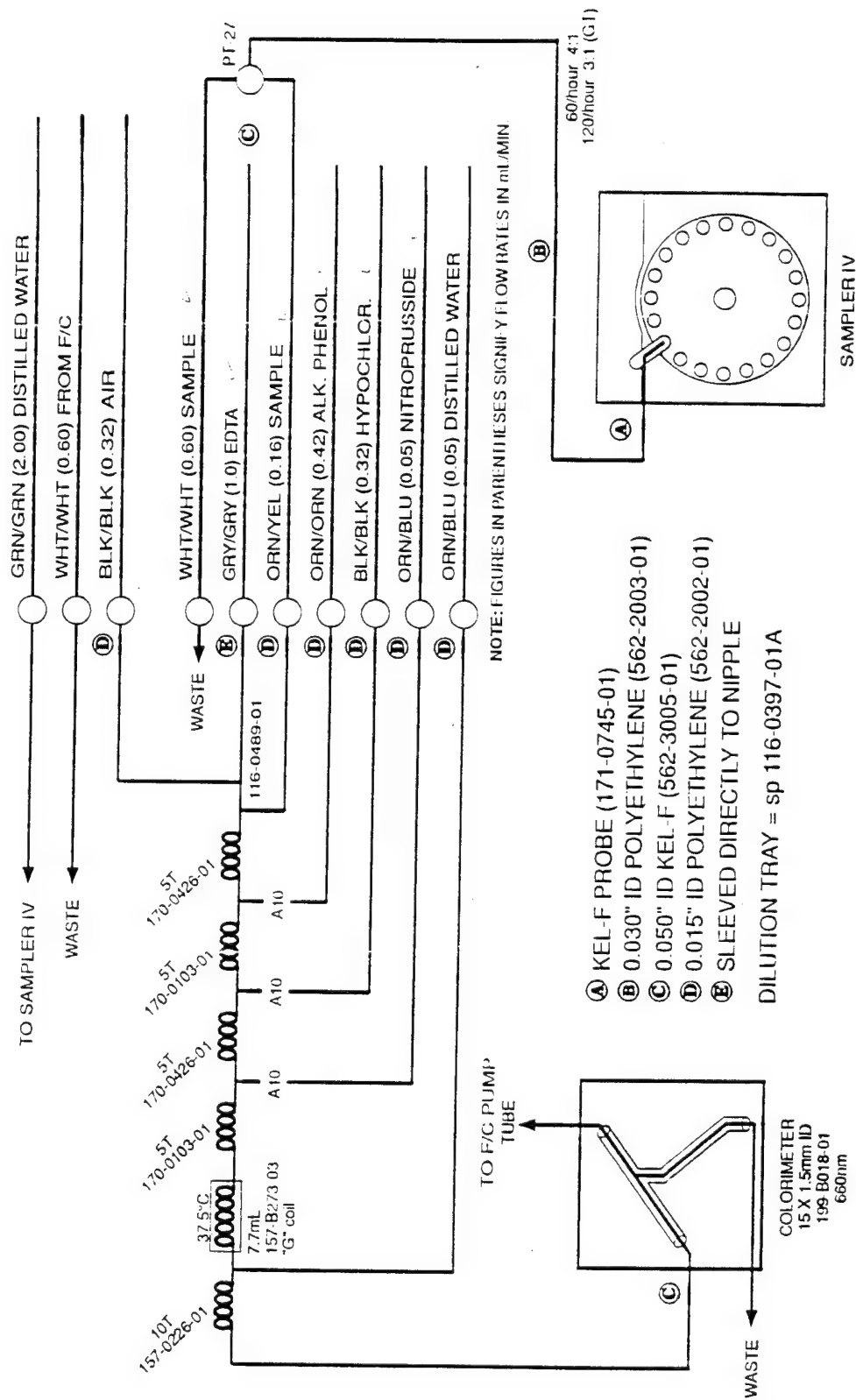
1.2 %

## Flow Diagram

1. If analysis is to be performed in a contaminated environment, scrub the air with 5N  $H_2SO_4$ .
2. Check that the appropriate harness and optical filters are secured in their proper positions.



Ammonia in water and wastewater  
 Manifold 116-D857-01  
 Harness number: 116-B346-01  
 Range: 0.02-3mg/L  $\text{NH}_3\text{-N}$





## **Appendix B-9 – Lab Procedures for Biochemical Oxygen Demand (BOD)**



**Table of Contents – Appendix B-9**

<b>Documents</b>
<b>TVA Lab Procedure: BOD</b>
<b>Biochemical Oxygen Demand: Method 405.1 (5 day, 20°C)</b>



# TENNESSEE VALLEY AUTHORITY

NO.: AP-0032

## CHEMICAL AND ENVIRONMENTAL ANALYSIS SECTION

TITLE: BIOCHEMICAL OXYGEN DEMAND, 5-DAY

Signature	Title	Date
Prepared by: <i>Sammie C. Smith</i> Sammie C. Smith	Research Chemist	12/16/91
Concurred: <i>William J. Rogers</i> William J. Rogers	QA/QC Coordinator	12/17/91
Concurred:		
Concurred:		
Concurred:		
Concurred:		
Approved: <i>B. Paul Bernauer</i> B. Paul Bernauer	Supervisor, Chemical and Environmental Analysis Section	12-17-91

REVISION	RO	R1	R2		
CONTROL DATE:	05/31/90	01/04/91	12/20/91		

COPY NO.: \_\_\_\_\_ HAS BEEN ISSUED TO HOLDER ON \_\_\_\_\_



FORMAT FOR RCFs

1.0 Reason for Change

2.0 Changes This Revision

<u>Page</u>	<u>Action</u>
15	Replace

3.0 Summary of Changes

<u>Page</u>	<u>Action</u>	<u>Revision</u>	<u>Date</u>
15	Replace 7.5	02	12/20/91

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TITLE: BIOCHEMICAL OXYGEN DEMAND, 5-DAY		NO.: AP-0032	PAGE: 1 of 18
		DATE: 12/20/91	

1.0      PURPOSE

        This procedure provides a method for determining the relative oxygen requirements of municipal and industrial wastewater.

2.0      SCOPE

        The Biochemical Oxygen Demand (BOD<sub>5</sub>) test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at 20°C for five days.

3.0      SUMMARY

        The sample of waste, or an appropriate dilution, is incubated for five days at 20°C in the dark. The reduction in dissolved oxygen (DO) concentration during the incubation period yields a measure of the (BOD<sub>5</sub>).

4.0      REFERENCES

4.1      U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastewater, EPA-600/4-79-020, pp. 405.1-1 - 405.1-2, 1983.

4.2      American Public Health Association, Standard Methods For The Examination of Water and Wastewater, Seventeenth Edition, pp. 5-2 - 5-10, 1989.

5.0      RESPONSIBILITIES

5.1      The Laboratory Supervisor, or his designee, shall ensure that this procedure is followed during the analysis of all samples for (BOD<sub>5</sub>).



PAGE:	2 of 18	NO.:	AP-0032	TITLE:	BIOCHEMICAL OXYGEN DEMAND, 5-DAY
		DATE:	12/20/91		

5.2 The Laboratory Shift Supervisor/Laboratory Group Leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure.

5.3 The analyst shall follow this procedure and report any abnormal results or nonconformance to the Laboratory Shift Supervisor/Laboratory Group Leader.

6.0 PROCEDURE/REQUIREMENTS

6.1 Prerequisites

6.1.1 Store a container (no more than two-thirds full, with cotton plug not capped) of deionized distilled water, free of organic matter, in the incubator set at 20°C for approximately 24 hours before beginning the BOD5 test.

6.1.2 Samples containing caustic alkalinity or acidity: Neutralize samples to pH of 6.5 to 7.5 with a solution of sulfuric acid (H2SO4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%.

6.1.3 Samples supersaturated with DO: Samples containing more than 9 mg DO/L at 20°C may be encountered in cold waters or in waters where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturated at 20°C by bringing sample to about 20°C in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

6.1.4 Sample temperature adjustment: Bring samples to 20 ± 1°C before making dilutions.



TITLE: BIOCHEMICAL OXYGEN DEMAND, 5-DAY	NO.: AP-0032 DATE: 12/20/91	PAGE: 3 of 18
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## 6.2 Limitations and Actions

6.2.1 Grab samples: If analysis is begun within two hours of collection, cold storage is unnecessary. If analysis is not started within 2 hours of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 hours of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case, start analysis more than 24 hours after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 hours of collection.

6.2.2 Composite samples: Keep samples at or below 4°C during compositing. Limit compositing period to 24 hours. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

## 6.3 Requirements

### 6.3.1 Apparatus/Equipment

NOTE: All new and used glassware must be thoroughly cleaned with a detergent, rinsed with deionized distilled water, and allowed to drain before use.

6.3.1.1 Incubator: Thermostatically controlled at  $20 \pm 1^\circ\text{C}$ .

6.3.1.2 BOD bottles: 300 mL with stoppers and caps.

6.3.1.3 Graduated cylinders: Various sizes.

6.3.1.4 Volumetric pipets: Various sizes.

6.3.1.5 Dissolved oxygen meter with BOD probe.



PAGE: 4 of 18	NO.: AP-0032 DATE: 12/20/91	TITLE: BIOCHEMICAL OXYGEN DEMAND, 5-DAY
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6.3.1.6 Analytical balance: Capable of weighing 0.1 mg.

6.3.2 Reagents and Standards

NOTE: Discard any of the following reagents if there is any sign of biological growth in the stock bottle.

6.3.2.1 Buffer nutrient pillows: 3-mL size prepares 3 liters of dilution water. May be obtained from Hach Company (Catalog No. 14861-98).

6.3.2.2 Phosphate buffer solution: Dissolve 8.5-grams potassium phosphate, monobasic ( $\text{KH}_2\text{PO}_4$ ), 21.75-grams potassium phosphate, diabasic ( $\text{KHPO}_4$ ), 33.4-grams sodium phosphate, diabasic ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ), and 1.7-grams ammonium chloride ( $\text{NH}_4\text{Cl}$ ) in about 500 mL of deionized distilled water and dilute with deionized distilled water to 1 liter. Mix well. The pH should be 7.2 without further adjustment.

6.3.2.3 Magnesium sulfate solution: Dissolve 22.5 grams of magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) in deionized distilled water and dilute to 1 liter.

6.3.2.4 Calcium chloride solution: Dissolve 27.5 grams of calcium chloride ( $\text{CaCl}_2$ ) in deionized distilled water and dilute to 1 liter.

6.3.2.5 Ferric chloride solution: Dissolve 0.25 grams of ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) in deionized distilled water and dilute to 1 liter.

6.3.2.6 Sulfuric acid solution -  $\text{H}_2\text{SO}_4$  -1N: Slowly and while stirring, add 28 mL of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to deionized distilled water. Dilute to 1 liter.



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6.3.2.7 Sodium hydroxide solution - NaOH-1N: Dissolve 40 grams of sodium hydroxide (NaOH) in deionized distilled water. Dilute to 1 liter.

6.3.2.8 Sodium sulfite solution: Dissolve 1.575 grams of Na<sub>2</sub>SO<sub>3</sub> in 1000-mL deionized distilled water. This solution is not stable; prepare daily.

6.3.2.9 Glucose-glutamic acid solution: Dry reagent-grade glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) and reagent-grade glutamic acid {C<sub>3</sub>H<sub>5</sub>NH<sub>2</sub>(COOH)<sub>2</sub>} at 103°C for one hour. Add 150-mg glucose and 150-mg glutamic acid to deionized distilled water and dilute to 1 liter. Prepare fresh immediately before use.

6.3.2.10 Polyseed solution: Place the contents of one Polyseed capsule (discard the gelatin capsule) in 500 mL of dilution water (6.5.1.2). Aerate and stir the Polyseed solution for 60 minutes. CONTINUE to stir and aerate the Polyseed solution while adding to BOD bottles.

NOTE 1: For best results, the Polyseed solution should be used within 6 hours of rehydration.

NOTE 2: Bran, which acts as the carrier for the micro-organisms, will neither dissolve, nor will it inhibit microbial activity.

NOTE 3: Polyseed is a blend of bacteria having a broad spectrum of capabilities for degradation of both industrial and municipal wastes. May be obtained from Baxter Scientific Products (Catalog No. W2802-1).

6.3.2.11 Sulfuric acid solution - H<sub>2</sub>SO<sub>4</sub> - 1 + 50: Slowly and while stirring, add 10 mL of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) to deionized distilled water. Dilute to 500 mL.



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6.3.2.12 Potassium iodide solution - KI: Dissolve 100 grams of potassium iodide (KI) in deionized distilled water. Dilute to 1 liter.

6.3.2.13 Starch indicator solution: To 5 grams of starch (potato, arrowroot, or soluble), add a little cold deionized distilled water and grind in a mortar to a thin paste. Pour 1 liter of boiling deionized distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25-grams salicylic acid or 4-grams zinc chloride.

6.4 Calibration

NOTE 1: Replace probe membrane if a large bubble forms in the electrolyte or if the membrane becomes fouled or damaged. Inspect the gold cathode when changing the membrane; it should be right and untarnished.

NOTE 2: Calibrate before each use.

6.4.1 Dissolved oxygen meter: YSI model 58 with YSI BOD bottle probe model 5720A.

6.4.1.1 Set the function switch to % mode.

6.4.1.2 Place the BOD probe in a BOD bottle containing about one inch of water to provide a 100% relative humidity calibration environment.

NOTE: The highest accuracy of measurement is achieved when the probe is zeroed and calibrated at a temperature as close as possible to the temperature of the sample to be measured.

6.4.1.3 Set the function switch to ZERO and readjust display to read 0.00. Switch back to % air saturation mode.



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6.4.1.4 When the display reading has stabilized, unlock the O2 CALIB control locking ring and adjust the display to the CALIB VALUE indicated in the PRESSURE/ALTITUDE chart in Attachment 2 (also printed in the instructions on the back of the meter). Relock the locking ring to prevent inadvertent changes.

6.4.1.5 Record the barometric pressure and the CALIB VALUE on the analysis worksheet (Attachment 1).

#### 6.5 Procedure Instructions

NOTE: Begin analysis within 6 hours of collection.

##### 6.5.1 Preparation of dilution water

6.5.1.1 Approximately 24 hours before analysis is to begin, place a desired volume of deionized distilled water, free of organic matter, in a suitable container (clean plastic or glass, no more than two-thirds full, with cotton plug not capped) in the incubator set at 20°C. This water will be used for dilution water. Add oxygen by shaking for at least 30 seconds.

6.5.1.2 Dilution water: Add one 3-mL buffer nutrient pillow (6.3.2.1) to deionized water (6.5.1.1) for each 3 liters of dilution water. [Alternately add 1 mL of each of the following solutions for each liter of dilution water: phosphate buffer solution (6.3.2.2), magnesium sulfate solution (6.3.2.3), calcium chloride solution (6.3.2.4), and ferric chloride solution (6.3.2.5)]. Mix and add oxygen by shaking for at least 30 seconds. Before using, bring dilution water temperature to 20°C.



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6.5.2 Sample dechlorination

6.5.2.1 Adjust sample pH if necessary between 6.5 and 7.5 using 1N H<sub>2</sub>SO<sub>4</sub> (6.3.2.6) or 1N NaOH (6.3.2.7).

6.5.2.2 Aliquot 100 mL of neutralized sample into a white porcelain casserole.

6.5.2.3 Add 10 mL of 1 + 50 sulfuric acid (6.3.2.11), 10 mL of potassium iodide solution (6.3.2.12), about 1 mL of starch solution (6.3.2.13), and titrate with sodium sulfite solution (6.3.2.8) to the starch-iodine end point.

6.5.2.4 Determine the volume of neutralized sample needed to perform the BOD test.

6.5.2.5 Add to neutralized sample, the relative volume of sodium sulfite solution (6.3.2.8) determined by the above test plus a 2-mL excess (for example: to dechlorinate 1 liter of neutralized sample, add 10 times the sodium sulfide titration volume plus 2 mL). Mix, and after 10 to 20 minutes check sample for residual chloride.

NOTE: Excess sodium sulfite exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.

6.5.3 Sample dilution technique

NOTE 1: For domestic wastewater, unchlorinated or otherwise undisinfected effluents from biological waste treatment plants, and surface waters receiving wastewater discharges containing satisfactory microbial populations, the addition of seeding material to the samples (6.5.3.9) and the glucose-glutamic acid check samples (6.5.3.5), and determining the seed BOD (6.5.3.11) is not necessary.

NOTE 2: Dilutions that result in a residual DO of at least 1 mg/L and a DO uptake of at least 2 mg/L after 5 days incubation produce the most reliable results. Make several dilutions of prepared sample to obtain DO uptake in this range.



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- 6.5.3.1 Adjust sample temperature to  $20 \pm 1^{\circ}\text{C}$  before making dilutions.
- 6.5.3.2 Label the BOD bottles for sample identification and record bottle number on analysis worksheet.
- 6.5.3.3 Dilution water blank: Fill one BOD bottle to overfill with the aerated dilution water (6.5.1.2). Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.
- 6.5.3.4 Seeded dilution water blank: Fill one BOD bottle about one-half full with aerated dilution water (6.5.1.2). Add 2 mL of polyseed solution (6.3.2.10) and finish filling with aerated dilution water. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles. The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L.
- 6.5.3.5 Select a range of dilutions that result in a residual DO of at least 1 mg/L and DO uptake of at least 2 mg/L after 5 days incubation.
- 6.5.3.6 Using a wide-tip volumetric pipet, add the desired dechlorinated sample volume to individual BOD bottles. Add the sample slowly without agitation by running the sample down the side of the bottle.



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6.5.3.7 Add 2 mL of the polyseed solution (6.3.2.10) to each bottle slowly without agitation by running the solution down the side of the bottle.

6.5.3.8 Fill bottles with enough dilution water so that insertion of stopper will displace all air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.3.9 Seed control: Add 25 mL of the polyseed solution (6.3.2.10) to 3 BOD bottles slowly without agitation by running the solution down the side of the bottle. Fill the bottles with enough dilution water so that insertion of stopper will displace air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.4 Glucose-glutamic acid check samples

NOTE 1: The glucose-glutamic acid check is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique.

NOTE 2: The acceptable range for results on the glucose-glutamic acid check is between 163 mg/L and 237 mg/L. Samples must be recollected for BOD analysis if the glucose-glutamic acid check falls outside this range.

6.5.4.1 Pipet 6 mL of the glucose-glutamic acid solution (6.3.2.9) and 2 mL of the polyseed solution (6.3.2.10) into 3 BOD bottles.



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- 6.5.4.2 Fill bottles with enough dilution water so that insertion of stopper will displace all air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.
- 6.5.5 Determination of initial DO
- 6.5.5.1 Calibrate the DO meter (see 6.4).
- 6.5.5.2 Determine the DO of each sample, check samples, seed control, and dilution water. Insert the BOD probe slowly into the BOD bottle to prevent trapping air bubbles. Turn probe stirrer on. Let sample stir at least 30 seconds before taking readings.
- 6.5.5.3 Turn the DO meter function switch to 02 ZERO and adjust if necessary.
- 6.5.5.4 Turn the DO meter function switch to TEMP and record the temperature on the analysis worksheet.
- 6.5.5.5 Turn the DO meter function switch to the 0.01 mg/L position and read the DO. Record the DO on the analysis worksheet.
- 6.5.5.6 Remove the BOD probe from the bottle slowly to prevent drawing air into the sample. Replace any displaced contents with dilution water.
- 6.5.5.7 Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.



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6.5.5.8 Water-seal by filling the BOD bottle neck completely with dilution water.

6.5.5.9 Cap the bottle with the special BOD cap.

6.5.5.10 Incubate the sample dilutions, check samples, and dilution water blank at  $20 \pm 1^{\circ}\text{C}$  for 5 days.

6.5.6 Determination of final DO

After 5 days remove all bottles from the incubator and determine the final DO of the sample dilutions, check samples, seed controls, and dilution water blanks by performing steps 6.5.5.1 through 6.5.5.5.

NOTE: The DO uptake of the dilution water blank should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

6.5.7 Glassware cleaning

6.5.7.1 Wash all BOD bottles, stoppers, and caps in hot, soapy water. Rinse several times with deionized distilled water.

6.5.7.2 Invert and allow to drain until dry. When dry, stopper and store them in the incubator until needed.

6.6 Calculations and Recording Data

NOTE 1: If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

NOTE 2: In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated above. If the dilution water does not meet these criteria, proper corrections are difficult and results become questionable.



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6.6.1 
$$\text{BOD}_5, \text{ mg/L} = \frac{\{(D1 - D2) - [(B1 - B2) \times f]\} \times 300}{A}$$

where:

D1 = DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5-day incubation at 20°C, mg/L

B1 = DO of seed control before incubation, mg/L

B2 = DO of seed control after 5-day incubation at 20°C, mg/L

f = Ratio of seed in diluted sample to seed in seed control  
= (% seed in diluted sample)/(% seed in seed control)

300 = Volume of BOD bottles, mL

A = Volume of sample used, mL

#### 6.6.2 Data Recording

Record all data and calculations on the appropriate worksheet (Attachment 1).

### 7.0 QUALITY ASSURANCE PROVISION

#### 7.1 Responsibility of Inspection

7.1.1 The analysts shall inspect all numerical data reasonableness and shall observe the DO meter, the dilutions, and other phenomena associated with the procedure for unusual occurrences.

7.1.2 The Shift Supervisor, or the Control Laboratory Supervisor or his designee, shall inspect the results of each analysis.

7.1.3 The Control Laboratory Supervisor, or his designee, shall inspect the results of the procedure on a regular basis.



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## 7.2 Acceptable Criteria

Critical measurements are made in the following steps:

6.5.1.1-- Addition of oxygen to deionized distilled water to  
be used for dilution water

6.5.1.2-- Preparation of dilution water

6.5.2.1-- Sample pH adjustment

6.5.2.3-- Determination of residual chloride

6.5.2.5-- Dechlorination of sample to be used for BOD  
determination

6.5.3.6-- Pipeting sample

6.5.3.7-- Adding polyseed to samples

6.5.3.8-- Filling sample bottles with dilution water

6.5.3.9-- Preparation of seed control

6.5.4.1-- Pipeting glucose-glutamic acid solution

6.5.4.2-- Filling check samples bottles with dilution water

6.5.5.1-- Calibration of DO meter

6.5.5.2-- Determining and recording the initial DO

6.5.5.10- Incubation for 5 days

6.5.6 -- Determining and recording the final DO

6.6 -- Calculations and recording data

## 7.3 Material Monitoring

All reagents shall be of ACS reagent-grade quality.

## 7.4 Equipment Monitoring

7.4.1 Record incubator temperature daily on Record Of Temperatures,  
Standards Recovery, And Blanks Report Sheet (Attachment 3).

7.4.2 Observe condition of BOD probe membrane before reading DO.



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7.5	Certification  This procedure shall be certified by the triplicate analysis of a certified demand QC sample. The average results and each individual results shall be within the 95% confidence interval of the QC sample.		
8.0	<u>SAFETY</u>  General laboratory safety rules shall be observed.		
9.0	<u>NOTES</u>  None		
10.0	<u>ATTACHMENTS AND APPENDICES</u>		
10.1	Attachment 1--BOD Analysis Worksheet.		
10.2	Attachment 2--Calibration Values for Various Atmospheric Pressures and Altitudes.		
10.3	Attachment 3--Record Of Temperatures, Standards Recovery, And Blanks Report Sheet.		



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ATTACHMENT 1

## BOD ANALYSIS WORKSHEET

SAMPLE IDENTIFICATION: \_\_\_\_\_

### BOD 5-DAY (Method 405.1)

DO METER Calib.: Bar. Press. = _____ mm Hg;	Calib. Value = _____
SAMPLE Collected: Date: Began _____	Time: Began _____
End _____	End _____
INCUBATION: Date: Began _____	Time: Began _____
5-DAY @ 20°C End _____	End _____
SAMPLE: Temp. = _____ °C; pH = _____ @ _____ °C; DO = _____ mg/l	COMPOSITE _____ GRAB _____

### SAMPLE DECHLORINATION

Na <sub>2</sub> SO <sub>3</sub> used to titrate 100 mL sample, mL	A
Na <sub>2</sub> SO <sub>3</sub> to dechlorinate 1 L of sample, mL, [(A x 10) + 2 mL]	B
Na <sub>2</sub> SO <sub>3</sub> to titrate 100 mL sample after addition of "B", mL	C

### SEED BOD5

Bottle Number								
Volume seed analyzed, mL	D							
Initial temperature, °C								
Initial DO, mg/L	E							
Final temperature, °C								
Final DO, mg/L	F							
DO depletion, mg/L, (E-F)	G							

### SAMPLE BOD5

Bottle Number								
Volume sample analyzed, mL	H	DW	DW + seed					
Volume seed added, mL	J	xxxx	2	2	2	2	2	2
Initial temperature, °C								
Initial DO, mg/L	K							
Final temperature, °C								
Final DO, mg/L	L							
DO depletion, mg/L, (K-L)	M							
Seed correction (GxJ/D)	N							
BOD, (M-N)x(300/H)								

### GLUCOSE-GLUTAMIC ACID CHECK

Bottle Number								
Volume check solution, mL	P							
Volume seed added, mL	R	2	2	2	2	2	2	2
Initial temperature, °C								
Initial DO, mg/L	S							
Final temperature, °C								
Final DO, mg/L	T							
DO depletion, mg/L, (S-T)	U							
BOD, (U-(GxR/D))x(300/P)								

Analyzed by \_\_\_\_\_  
Checked by \_\_\_\_\_

FORMBOD5



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ATTACHMENT 2

PRESSURE			ALTITUDE		CALIB. VALUE
Inches Hg	mm Hg	kPa	FT.	m	
30.23	768	102.3	-276	-84	101
29.92	760	101.3	0	0	100
29.61	752	100.3	278	85	99
29.33	745	99.3	558	170	98
29.02	737	98.3	841	256	97
28.74	730	97.3	1126	343	96
28.43	722	96.3	1413	431	95
28.11	714	95.2	1703	519	94
27.83	707	94.2	1995	608	93
27.52	699	93.2	2290	698	92
27.24	692	92.2	2587	789	91
26.93	684	91.2	2887	880	90
26.61	676	90.2	3190	972	89
26.34	669	89.2	3496	1066	88
26.02	661	88.2	3804	1160	87
25.75	654	87.1	4115	1254	86
25.43	646	86.1	4430	1350	85
25.12	638	85.1	4747	1447	84
24.84	631	84.1	5067	1544	83
24.53	623	83.1	5391	1643	82
24.25	616	82.1	5717	1743	81
23.94	608	81.1	6047	1843	80
23.62	600	80.0	6381	1945	79
23.35	593	79.0	6717	2047	78
23.03	585	78.0	7058	2151	77
22.76	578	77.0	7401	2256	76
22.44	570	76.0	7749	2362	75
22.13	562	75.0	8100	2469	74
21.85	555	74.0	8455	2577	73
21.54	547	73.0	8815	2687	72
21.26	540	71.9	9178	2797	71
20.94	532	70.9	9545	2909	70
20.63	524	69.9	9917	3023	69
20.35	517	68.9	10293	3137	68
20.04	509	67.9	10673	3253	67
19.76	502	66.9	11058	3371	66

Calibration Values for various atmospheric pressures and altitudes.  
Normal barometric variations are equivalent to  $\sim \pm 500$  feet at sea level.



ATTACHMENT 3

IWA CONTROL LABORATORY

RECORD OF TEMPERATURES, STANDARDS RECOVERY, AND BLANKS

Date	Oven Temp. °F	BOD INCUB. °C	O & G BATH °C	Std. - % Recovery				Oil & Grease	Blanks (mg / liter)				
				Titration		Electrode			Titration		Electrode		
				T.K.N.	NH3-N	T.K.N.	NH3-N		T.K.N.	NH3-N	T.K.N.	NH3-N	
1													
2													
3													
4													
5													
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# BIOCHEMICAL OXYGEN DEMAND

## Method 405.1 (5 Days, 20°C)

STORET NO. 00310  
Carbonaceous 80082

1. Scope and Application
  - 1.1 The biochemical oxygen demand (BOD) test is used for determining the relative oxygen requirements of municipal and industrial wastewaters. Application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen resources of the receiving water. Data from BOD tests are used for the development of engineering criteria for the design of wastewater treatment plants.
  - 1.2 The BOD test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at 20°C for a specified time period (often 5 days). The actual environmental conditions of temperature, biological population, water movement, sunlight, and oxygen concentration cannot be accurately reproduced in the laboratory. Results obtained must take into account the above factors when relating BOD results to stream oxygen demands.
2. Summary of Method
  - 2.1 The sample of waste, or an appropriate dilution, is incubated for 5 days at 20°C in the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.
3. Comments
  - 3.1 Determination of dissolved oxygen in the BOD test may be made by use of either the Modified Winkler with Full-Bottle Technique or the Probe Method in this manual.
  - 3.2 Additional information relating to oxygen demanding characteristics of wastewaters can be gained by applying the Total Organic Carbon and Chemical Oxygen Demand tests (also found in this manual).
  - 3.3 The use of 60 ml incubation bottles in place of the usual 300 ml incubation bottles, in conjunction with the probe, is often convenient.
4. Precision and Accuracy
  - 4.1 Eighty-six analysts in fifty-eight laboratories analyzed natural water samples plus an exact increment of biodegradable organic compounds. At a mean value of 2.1 and 175 mg/l BOD, the standard deviation was  $\pm 0.7$  and  $\pm 26$  mg/l, respectively (EPA Method Research Study 3).
  - 4.2 There is no acceptable procedure for determining the accuracy of the BOD test.

Approved for NPDES CBOD: pending approval for Section 304(h), CWA

Issued 1971

Editorial revision 1974



5. References

- 5.1 The procedure to be used for this determination is found in:  
Standard Methods for the Examination of Water and Wastewater, 15th  
Edition, p. 483, Method 507 (1980).
- 5.2 Young, J. C., "Chemical Methods for Nitrification Control," J. Water  
Poll. Control Fed., 45, p. 637 (1973).



**Appendix B-10 – Lab Procedures for Residues (Filterable, Non-Filterable, Total)**



## Table of Contents – Appendix B-10

Documents
QC Document – Residue
Residue, Filterable: Method 160.1 (Gravimetric, Dried at 180°C)
Residue, Filterable: Method 160.2 (Gravimetric, Dried at 103-105°C)
Residue, Total: Method 160.3 (Gravimetric, Dried at 103-105°C)



Residue	Method 160.1	Residue, Filterable
	Method 160.2	Residue, Non-Filterable
	Method 160.3	Residue, Total

#### 1.0 Procedure

Perform residue analysis in accordance with any or all of procedures 160.1, 160.2, or 160.3 as requested.

#### 2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

#### 3.0 Quality Control Samples

None



## RESIDUE, FILTERABLE

### Method 160.1 (Gravimetric, Dried at 180°C)

STORET NO. 70300

1. Scope and Application
  - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
  - 1.2 The practical range of the determination is 10 mg/l to 20,000 mg/l.
2. Summary of Method
  - 2.1 A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180°C.
  - 2.2 If Residue, Non-Filterable is being determined, the filtrate from that method may be used for Residue, Filterable.
3. Definitions
  - 3.1 Filterable residue is defined as those solids capable of passing through a glass fiber filter and dried to constant weight at 180°C.
4. Sample Handling and Preservation
  - 4.1 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
  - 5.1 Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying, desiccation and rapid weighing.
  - 5.2 Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180°C to insure that all the bicarbonate is converted to carbonate.
  - 5.3 Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.
6. Apparatus
  - 6.1 Glass fiber filter discs, 4.7 cm or 2.1 cm, without organic binder. Reeve Angel type 934-AH, Gelman type A/E, or equivalent.
  - 6.2 Filter holder, membrane filter funnel or Gooch crucible adapter.
  - 6.3 Suction flask, 500 ml.
  - 6.4 Gooch crucibles, 25 ml (if 2.1 cm filter is used).
  - 6.5 Evaporating dishes, porcelain, 100 ml volume. (Vycor or platinum dishes may be substituted).
  - 6.6 Steam bath.
  - 6.7 Drying oven, 180°C  $\pm$  2°C.
  - 6.8 Desiccator.

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6.9 Analytical balance, capable of weighing to 0.1 mg.

7. Procedure

- 7.1 Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard washings.
- 7.2 Preparation of evaporating dishes: If Volatile Residue is also to be measured heat the clean dish to  $550 \pm 50^\circ\text{C}$  for one hour in a muffle furnace. If only Filterable Residue is to be measured heat the clean dish to  $180 \pm 2^\circ\text{C}$  for one hour. Cool in desiccator and store until needed. Weigh immediately before use.
- 7.3 Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 ml to the funnel by means of a 100 ml graduated cylinder. If total filterable residue is low, a larger volume may be filtered.
- 7.4 Filter the sample through the glass fiber filter, rinse with three 10 ml portions of distilled water and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.
- 7.5 Transfer 100 ml (or a larger volume) of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.
- 7.6 Dry the evaporated sample for at least one hour at  $180 \pm 2^\circ\text{C}$ . Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

8. Calculation

- 8.1 Calculate filterable residue as follows:

$$\text{Filterable residue, mg/l} = \frac{(A - B) \times 1,000}{C}$$

where:

A = weight of dried residue + dish in mg

B = weight of dish in mg

C = volume of sample used in ml

9. Precision and Accuracy

- 9.1 Precision and accuracy are not available at this time.

**Bibliography**

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 92. Method 208B. (1975).



## RESIDUE, NON-FILTERABLE

### Method 160.2 (Gravimetric, Dried at 103–105°C)

STORET NO. 00530

1. Scope and Application
  - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
  - 1.2 The practical range of the determination is 4 mg/l to 20,000 mg/l.
2. Summary of Method
  - 2.1 A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103–105°C.
  - 2.2 The filtrate from this method may be used for Residue, Filterable.
3. Definitions
  - 3.1 Residue, non-filterable, is defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103–105°C.
4. Sample Handling and Preservation
  - 4.1 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
  - 4.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
  - 5.1 Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.
  - 5.2 Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.
6. Apparatus
  - 6.1 Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.

**NOTE:** Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.
  - 6.2 Filter support: filtering apparatus with reservoir and a coarse (40–60 microns) fritted disc as a filter support.

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**NOTE:** Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

- 6.3 Suction flask.
  - 6.4 Drying oven, 103–105°C.
  - 6.5 Desiccator.
  - 6.6 Analytical balance, capable of weighing to 0.1 mg.
  - 7. Procedure
    - 7.1 Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103–105°C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.
    - 7.2 Selection of Sample Volume

For a 4.7 cm diameter filter, filter 100 ml of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm<sup>2</sup> of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.

**NOTE:** If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five ml increments for timing are suggested. Continue to record the time and volume increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.
    - 7.3 Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.
    - 7.4 Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected in 7.2 to the filter using a graduated cylinder. Remove all traces of water by continuing to apply vacuum after sample has passed through.
    - 7.5 With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.
- NOTE:** Total volume of wash water used should equal approximately 2 ml per cm<sup>2</sup>. For a 4.7 cm filter the total volume is 30 ml.



- 7.6 Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103–105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).
8. Calculations
- 8.1 Calculate non-filterable residue as follows:

$$\text{Non-filterable residue, mg/l} = \frac{(A - B) \times 1,000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = ml of sample filtered

9. Precision and Accuracy
- 9.1 Precision data are not available at this time.
- 9.2 Accuracy data on actual samples cannot be obtained.

#### Bibliography

1. NCASI Technical Bulletin No. 291, March 1977. National Council of the Paper Industry for Air and Stream Improvement, Inc., 260 Madison Ave., NY.



## RESIDUE, TOTAL

### Method 160.3 (Gravimetric, Dried at 103–105°C)

STORET NO. 00500

1. Scope and Application
  - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
  - 1.2 The practical range of the determination is from 10 mg/l to 20,000 mg/l.
2. Summary of Method
  - 2.1 A well mixed aliquot of the sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103–105°C.
3. Definitions
  - 3.1 Total Residue is defined as the sum of the homogenous suspended and dissolved materials in a sample.
4. Sample Handling and Preservation
  - 4.1 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
  - 5.1 Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
  - 5.2 Floating oil and grease, if present, should be included in the sample and dispersed by a blender device before aliquoting.
6. Apparatus
  - 6.1 Evaporating dishes, porcelain, 90 mm, 100 ml capacity. (Vycor or platinum dishes may be substituted and smaller size dishes may be used if required.)
7. Procedure
  - 7.1 Heat the clean evaporating dish to 103–105°C for one hour, if Volatile Residue is to be measured, heat at 550 ± 50°C for one hour in a muffle furnace. Cool, desiccate, weigh and store in desiccator until ready for use.
  - 7.2 Transfer a measured aliquot of sample to the pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven.
    - 7.2.1 Choose an aliquot of sample sufficient to contain a residue of at least 25 mg. To obtain a weighable residue, successive aliquots of sample may be added to the same dish.
    - 7.2.2 If evaporation is performed in a drying oven, the temperature should be lowered to approximately 98°C to prevent boiling and splattering of the sample.

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7.3 Dry the evaporated sample for at least 1 hour at 103–105°C. Cool in a desiccator and weigh. Repeat the cycle of drying at 103–105°C, cooling, desiccating and weighing until a constant weight is obtained or until loss of weight is less than 4% of the previous weight, or 0.5 mg, whichever is less.

8. Calculation

8.1 Calculate total residue as follows:

$$\text{Total residue, mg/l} = \frac{(A - B) \times 1,000}{C}$$

where:

A = weight of sample + dish in mg

B = weight of dish in mg

C = volume of sample in ml

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

**Bibliography**

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 91, Method 208A, (1975).



**Appendix B-11 – Lab Procedures for Temperature: Method 170.1**



## Table of Contents – Appendix B-11

Documents
QC Document – Temperature - Method 170.1
Temperature, Method 170.1 (Thermometric)



## Temperature - Method 170.1

### 1.0 Procedure

Perform temperature measurements in accordance with "Temperature" Method 170.1 (Thermometric) as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

### 3.0 Quality Control Samples

None



## TEMPERATURE

### Method 170.1 (Thermometric)

STORET NO. 00010

1. Scope and Application
  - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
  - 2.1 Temperature measurements may be made with any good grade of mercury-filled or dial type centigrade thermometer, or a thermistor.
3. Comments
  - 3.1 Measurement device should be routinely checked against a precision thermometer certified by the National Bureau of Standards.
4. Precision and Accuracy
  - 4.1 Precision and accuracy for this method have not been determined.
5. Reference
  - 5.1 The procedure to be used for this determination is found in:  
Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 125,  
Method 212 (1975).



**Appendix B-12 – Lab Procedures for Chemical Oxygen Demand (COD):  
Method Series 410 with Hach DR/3000**



## Table of Contents – Appendix B-12

Documents
QC Document – Chemical Oxygen Demand – Method Series 410 with Hach DR/3000
Oxygen Demand, Chemical DR/3000 Procedure Code 0.9 Range: 0-150 ml/g Reactor Digestion Method.
Oxygen Demand, Chemical DR/3000 Procedure Code 0.1 Range: 0-1,500 ml/g Reactor Digestion Method.
Oxygen Demand, Chemical DR/3000 Procedure Code 0.11 Range: 0-15,000 ml/g Reactor Digestion Method.



## Chemical Oxygen Demand- Method Series 410 with Hach DR/3000

### 1.0 Procedure

Perform Chemical Oxygen Demand on the Hach DR/3000 in accordance with manufacturer's instructions as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank and one quality control sample made from a sucrose solution. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



# OXYGEN DEMAND, CHEMICAL

DR/3000 Procedure Code 0.9

Range: 0-150 mg/L

Reactor Digestion Method\*

## Introduction

The Chemical Oxygen Demand (COD) test is used widely to estimate the amount of organic matter in wastewater. It is a measurement of the oxygen equivalent of the materials present in the wastewater that are subject to oxidation by a strong chemical oxidant, in this case dichromate.

In the reactor digestion method test, the COD procedure is greatly simplified. Small volumes of the water sample are pipetted into vials containing the premeasured reagents, including catalysts and chloride compensator. The vials are digested at 150°C for two hours and cooled; then the COD determination is made either with the spectrophotometer or by titration. This method has been approved for NPDES reporting purposes by the EPA.<sup>7</sup>

Both the titrimetric method and colorimetric method for determining Chemical Oxygen Demand (COD) are contained in this manual. The digestion procedure is needed for both methods, with the choice of the final measurement method left to the analyst. The colorimetric method is the simpler and quicker of the two. The titrimetric method should be used if turbidity or colored species remain after digestion.

### WARNING

*Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings on the labels and read the safety section of this manual. If you have questions or would like a reprint of the safety section, please contact Hach. In the procedure, hazardous substances appear in italic typeface wherever they are used in the test and deserve extra care in handling. It is always good practice to wear safety glasses when handling chemicals. Wash thoroughly if contact occurs. Follow instructions carefully.*

## Sampling and Storage

Collect samples in glass bottles if possible. Plastic bottles can be used only if they are known to be free of organic contamination. Biologically active samples should be tested as soon as possible. Samples containing solids should be well-mixed or homogenized to permit the removal of a representative aliquot. NOTE: The following storage instructions are necessary **only** when prompt analysis is impossible. Add 2.0-mL of *Sulfuric Acid*, ACS, for each liter or quart of sample using a glass serological pipet and pipet filler. Mix. Check the sample to be sure the pH is 2 or less. Add more acid if necessary. Samples preserved in this manner can be stored for seven days. Where significant amounts of preservatives are used, a volume correction should be made for the extra acid by dividing the total volume (sample + acid) by the sample volume and multiplying the test result by this factor.

## Digestion

1. Place approximately 500 mL of sample in a clean blender bowl and homogenize at high speed for two minutes. Blending the sample ensures a uniform distribution of suspended solids and thus improves the accuracy and reproducibility of test results.
2. Turn on the COD Reactor to preheat the 150°C. Wrap a towel around a *COD Digestion Reagent Vial* of the desired range and cautiously remove the cap. A towel also should be wrapped around the vials when re-tightening the caps to prevent injury. While holding a vial at a 45-degree angle, carefully pipet 2.00 mL of sample into the vial. Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run the COD test with vials that have had reagents spilled from them. See *Notes A and B*.

\*Jirka, A. M., and Carter, M. J., *Anal. Chem.*, 47, 8, 1397, (1975)



## Oxygen Demand, Chemical, Reactor Digestion (continued)

3. Wrap a towel around the vial and cautiously replace the cap. To avoid vapor loss and accidental spillage, be sure caps are well-tightened. Holding the vial by the cap in an empty sink, gently invert the vial several times to mix the contents. The vial will become very hot during mixing.
4. Place the vial in the preheated COD Reactor. The plastic shield should be in place on the heater block before vials are placed in the reactor.
5. Prepare a reagent blank by repeating Steps 2 through 4, substituting 2.00 mL of distilled or demineralized water in place of the water sample. Heat the vials for two hours at 150°C. Turn the reactor off and allow the vials to cool to 120°C or less (about 20 minutes). While the vials are still warm, invert each vial several times. Place vials in a cooling rack and allow to cool to the touch. Measure the COD. One blank from the same lot of vials must be run with each set of samples. The lot number appears to the right of the catalog number in the COD vial container. *See Notes C and D.*

### Procedure 0-150 mg/L COD Range

1. To begin the stored program for this test, initiate the program mode by making the following key strokes:

Press:



The STORED PROGRAM light will come on and a wavelength setting of 420.0 for COD testing will appear in the display. The SET WAVELENGTH and ZERO prompting lights will flash.

2. Turn the wavelength selector dial, located on the upper right corner of the instrument, to a setting of 420.

Press:



The SET WAVELENGTH prompting light will turn off and the display will read 0. The ZERO prompting light will continue to flash until the instrument is zeroed in Step 4.

3. Place the COD Vial Adapter in the cell holder of the spectrophotometer with the groove in the adapter facing the front of the instrument. Wipe the vial containing the reagent blank clean with a wet towel followed by a dry one. Insert the vial in the adapter with the Hach logo centered on the groove and cover with the light shield. *See Note E.*
4. This reagent blank is used to set the zero concentration point. Zero the instrument as follows:

Press:



The ZERO, CONCENTRATION and AUTO UPDATE lights will come on and a concentration value of 0 will appear in the display.

5. Wipe the vial containing the test sample with a wet towel followed by a dry one. Place the vial in the adapter, align the Hach logo with the groove, replace the light shield and read the mg/L COD from the display. *See Note F.*

### Notes

- A. The COD vials contain enough mercuric sulfate to complex more than 2000 mg/L chloride. As a general rule, accurate results cannot be obtained above this level. For higher chloride concentrations, dilute the sample so that the chloride concentration is less than 1000 mg/L. If this is not feasible due to low COD values, add 0.50 g *Mercuric Sulfate* to each COD vial prior to addition of sample and blank. Chloride con-



## Oxygen Demand, Chemical, Reactor Digestion (continued)

centrations of 8000 mg/L in the 0-150 mg/L COD vials and up to 4000 mg/L in the 0-1500 mg/L COD vials cause minimal errors.

- B. The mixture of potassium dichromate and sulfuric acid is sensitive to ultraviolet light. To avoid reagent deterioration during storage, keep reagent vials protected from all light sources and in a refrigerator if available. Store unused vials in the opaque shipping container. Under normal lighting conditions, the small amount of light that strikes the vials during the digestion period will not affect COD test results.
- C. Many wastewater samples containing easily oxidizable organic materials are digested completely in less than two hours. To use a reduced digestion period, colorimetrically read the sample vials (while still hot) at 15-minute intervals. When the reading remains unchanged, the sample may be considered completely digested.
- D. If a pure green color is obtained in the reacted sample, all the dichromate has been reduced to the chromic state. It will be necessary to repeat the digestion with a diluted sample. For best accuracy, dilute and repeat those determinations that approach 140-150 mg/L for low range and 1400-1500 mg/L COD for the high range.
- E. The blank has been found to be stable when kept in the dark. It can be monitored for decomposition by using the %T Mode and reading the color at 420 nm and 620 nm for the low and high range vials, respectively, vs. deionized water. Using a vial containing 5 mL of deionized water, press:



A transmittance value of 100.0 will appear on the display. Place the blank in the adapter and record the transmittance value. If the blank transmittance has changed more than 1-2 %T from its initial value, a new blank should be run. A new blank must be run when a different lot of vials is obtained.

- F. If the display flashes 165 for the 0-150 mg/L COD range or 1650 for the 0-1500 mg/L COD range, the upper limit of the precalibrated range has been exceeded and a sample dilution is needed. Flashing values of -3 or -25 for the low range and high range, respectively, indicate the sample has a high negative absorbance value relative to the blank. High negative values indicate a possible contamination of the blank or a procedure error by the user. Small negative values probably are due to small variations between vials or amounts of reagent added and should be treated as a concentration value of zero.

### Precision

In a single laboratory, using standard solutions of 100 mg/L COD and 1000 mg/L COD and two representative lots of reagent, a single operator obtained a standard deviation of  $\pm 1.4$  mg/L COD and  $\pm 14$  mg/L COD for 0-150 mg/L and 0-1500 mg/L ranges, respectively.

### Accuracy Check

Accuracy of the test can be checked by performing the procedure using 2 mL of 300 mg/L COD standard in place of the sample. The test result should be 300 mg/L COD. A 500-mg/L COD standard can be prepared by drying Potassium Acid Phthalate to a constant weight at 120°C, dissolving 425 mg in distilled water and diluting to 1 liter. Prepare fresh for each use. A low range COD standard can be prepared in the same manner. Dissolving 85 mg of dried potassium acid phthalate in 1 liter of distilled water will yield a 100-mg/L COD standard.



## Oxygen Demand, Chemical, Reactor Digestion (continued)

### Required Reagents and Apparatus for 0-150 mg/L COD Range

Cat. No.	Description	Unit
272-16	Demineralized Water	946 mL* (qt)
14515-36	Pipet, volumetric, Class A, 2.00 mL	each
16500-10	COD Reactor, 120-240 V	each
44799-00	COD Vial Adapter, DR/3000	each
18641-00	Test Tube Rack (2 recommended)	each
21258-25	<i>COD Digestion Reagent Vials, Low Range</i>	25

### Optional Reagents and Apparatus

315-34	Potassium Acid Phthalate	500 g
511-00	Measuring Spoon, 0.1 g	each
979-11	<i>Sulfuric Acid, ACS</i>	473 mL (pt)
1915-20	<i>Mercuric Sulfate</i>	28 g*
12186-31	Potassium Acid Phthalate, 300 mg/L COD	236 mL *(8 oz)
22539-31	Potassium Acid Phthalate, 1000 mg/L COD	236 mL (8 oz)

\*Larger sizes available



# OXYGEN DEMAND, CHEMICAL

## DR/3000 Procedure Code O.10

### Range: 0-1500 mg/L

### Reactor Digestion Method\*

#### Introduction

The Chemical Oxygen Demand (COD) test is used widely to estimate the amount of organic matter in wastewater. It is a measurement of the oxygen equivalent of the materials present in the wastewater that are subject to oxidation by a strong chemical oxidant, in this case dichromate.

In the reactor digestion method test, the COD procedure is greatly simplified. Small volumes of the water sample are pipetted into vials containing the premeasured reagents, including catalysts and chloride compensator. The vials are digested at 150°C for two hours and cooled; then the COD determination is made either with the spectrophotometer or by titration. This method has been approved for NPDES reporting purposes by the EPA.<sup>†</sup>

Both the titrimetric method and colorimetric method for determining Chemical Oxygen Demand (COD) are contained in this manual. The digestion procedure is needed for both methods, with the choice of the final measurement method left to the analyst. The colorimetric method is the simpler and quicker of the two. The titrimetric method should be used if turbidity or colored species remain after digestion.

#### WARNING

*Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings on the labels and read the safety section of this manual. If you have questions or would like a reprint of the safety section, please contact Hach. In the procedure, hazardous substances appear in italic typeface wherever they are used in the test and deserve extra care in handling. It is always good practice to wear safety glasses when handling chemicals. Wash thoroughly if contact occurs. Follow instructions carefully.*

#### Sampling and Storage

Collect samples in glass bottles if possible. Plastic bottles can be used only if they are known to be free of organic contamination. Biologically active samples should be tested as soon as possible. Samples containing solids should be well-mixed or homogenized to permit the removal of a representative aliquot. NOTE: The following storage instructions are necessary **only** when prompt analysis is impossible. Add 2.0-mL of *Sulfuric Acid*, ACS, for each liter or quart of sample using a glass serological pipet and pipet filler. Mix. Check the sample to be sure the pH is 2 or less. Add more acid if necessary. Samples preserved in this manner can be stored for seven days. Where significant amounts of preservatives are used, a volume correction should be made for the extra acid by dividing the total volume (sample + acid) by the sample volume and multiplying the test result by this factor.

#### Digestion

1. Place approximately 500 mL of sample in a clean blender bowl and homogenize at high speed for two minutes. Blending the sample ensures a uniform distribution of suspended solids and thus improves the accuracy and reproducibility of test results.
2. Turn on the COD Reactor to preheat the 150°C. Wrap a towel around a *COD Digestion Reagent Vial* of the desired range and cautiously remove the cap. A towel also should be wrapped around the vials when re-tightening the caps to prevent injury. While holding a vial at a 45-degree angle, carefully pipet 2.00 mL of sample into the vial. Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run the COD test with vials that have had reagents spilled from them. See *Notes A and B*.

\*Jirka, A. M., and Carter, M. J., *Anal. Chem.*, 47 (8), 1397 (1975)

†*Federal Register*, April 21, 1980, 45 (78) 26811-26812



## Oxygen Demand, Chemical, Reactor Digestion (continued)

3. Wrap a towel around the vial and cautiously replace the cap. To avoid vapor loss and accidental spillage, be sure caps are well-tightened. Holding the vial by the cap in an empty sink, gently invert the vial several times to mix the contents. The vial will become very hot during mixing.
4. Place the vial in the preheated COD Reactor. The plastic shield should be in place on the heater block before vials are placed in the reactor.
5. Prepare a reagent blank by repeating Steps 2 through 4, substituting 2.00 mL of distilled or demineralized water in place of the water sample. Heat the vials for two hours at 150°C. Turn the reactor off and allow the vials to cool to 120°C or less (about 20 minutes). While the vials are still warm, invert each vial several times. Place vials in a cooling rack and allow to cool to the touch. Measure the COD. One blank from the same lot of vials must be run with each set of samples. The lot number appears to the right of the catalog number in the COD vial container. See *Notes C and D*.

### Procedure 0-1500 mg/L COD Range

1. To begin the stored program for this test, initiate the program mode by making the following key strokes:

Press:



The STORED PROGRAM light will come on and a wavelength setting of 620.0 for COD testing will appear in the display. The SET WAVELENGTH and ZERO prompting lights will flash.

2. Turn the wavelength selector dial, located on the upper right corner of the instrument, to a setting of 620.

Press:



The SET WAVELENGTH prompting light will turn off and the display will read 0. The ZERO prompting light will continue to flash until the instrument is zeroed in Step 4.

3. Place the COD Vial Adapter in the cell holder of the spectrophotometer with the groove in the adapter facing the front of the instrument. Wipe the vial containing the reagent blank clean with a wet towel followed by a dry one. Insert the vial containing the reagent blank in the adapter with the Hach logo centered on the groove and cover with the light shield. See *Note E*.
4. This reagent blank is used to set the zero concentration point. Zero the instrument as follows:

Press:



The ZERO, CONCENTRATION and AUTO UPDATE lights will come on and a concentration value of 0 will appear in the display.

5. Wipe the vial containing the test sample clean with a wet towel followed by a dry one. Place the vial in the adapter, align the Hach logo with the groove, replace the light shield and read the mg/L COD from the display. See *Note F*.

### Notes

- A. The COD vials contain enough mercuric sulfate to complex more than 2000 mg/L chloride. As a general rule, accurate results cannot be obtained above this level. For higher chloride concentrations, dilute the sample so that the chloride concentration is less than 1000 mg/L. If this is not feasible due to low COD



## Oxygen Demand, Chemical, Reactor Digestion (continued)

values, add 0.50 g *Mercuric Sulfate* to each COD vial prior to addition of sample and blank. Chloride concentrations of 8000 mg/L in the 0-150 mg/L COD vials and up to 4000 mg/L in the 0-1500 mg/L COD vials cause minimal errors.

- B. The mixture of potassium dichromate and sulfuric acid is sensitive to ultraviolet light. To avoid reagent deterioration during storage, keep reagent vials protected from all light sources and in a refrigerator if available. Store unused vials in the opaque shipping container. Under normal lighting conditions, the small amount of light that strikes the vials during the digestion period will not affect COD test results.
- C. Many wastewater samples containing easily oxidizable organic materials are digested completely in less than two hours. To use a reduced digestion period, colorimetrically read the sample vials (while still hot) at 15-minute intervals. When the reading remains unchanged, the sample may be considered completely digested.
- D. If a pure green color is obtained in the reacted sample, all the dichromate has been reduced to the chromic state. It will be necessary to repeat the digestion with a diluted sample. For best accuracy, dilute and repeat those determinations that approach 140-150 mg/L for low range and 1400-1500 mg/L COD for the high range.
- E. The blank has been found to be stable when kept in the dark. It can be monitored for decomposition by using the %T Mode and reading the color at 420 nm and 620 nm for the low and high range vials, respectively, vs. deionized water. Using a vial containing 5 mL of deionized water, press:



A transmittance value of 100.0 will appear on the display. Place the blank in the adapter and record the transmittance value. If the blank transmittance has changed more than 1-2 %T from its initial value, a new blank should be run. A new blank must be run when a different lot of vials is obtained.

- F. If the display flashes 165 for the 0-150 mg/L COD range or 1650 for the 0-1500 mg/L COD range, the upper limit of the precalibrated range has been exceeded and a sample dilution is needed. Flashing values of -3 or -25 for the low range and high range, respectively, indicate the sample has a high negative absorbance value relative to the blank. High negative values indicate a possible contamination of the blank or a procedure error by the user. Small negative values probably are due to small variations between vials or amounts of reagent added and should be treated as a concentration value of zero.

### Precision

In a single laboratory, using standard solutions of 100 mg/L COD and 1000 mg/L COD and two representative lots of reagent, a single operator obtained a standard deviation of  $\pm 1.4$  mg/L COD and  $\pm 14$  mg/L COD for 0-150 mg/L and 0-1500 mg/L ranges, respectively.

### Accuracy Check

Accuracy of the test can be checked by performing the procedure using 2 mL of 300 mg/L COD standard in place of the sample. The test result should be 300 mg/L COD. A 500-mg/L COD standard can be prepared by drying Potassium Acid Phthalate to a constant weight at 120°C, dissolving 425 mg in distilled water and diluting to 1 liter. Prepare fresh for each use. A low range COD standard can be prepared in the same manner. Dissolving 85 mg of dried potassium acid phthalate in 1 liter of distilled water will yield a 100-mg/L COD standard.



## Oxygen Demand, Chemical, Reactor Digestion (continued)

### Required Reagents and Apparatus for 0-1500 mg/L COD Range

272-16	Demineralized Water	946 mL* (qt)
14515-36	Pipet, volumetric, Class A, 2.00 mL	each
16500-10	COD Reactor, 120-240 V	each
44799-00	COD Vial Adapter, DR/3000	each
18641-00	Test Tube Rack (2 recommended)	each
21259-25	<i>COD Digestion Reagent Vials, High Range</i>	25

### Optional Reagents and Apparatus

315-34	Potassium Acid Phthalate	500 g
511-00	Measuring Spoon, 0.1 g	each
979-11	<i>Sulfuric Acid, ACS</i>	473 mL (pt)
1915-20	<i>Mercuric Sulfate</i>	28 g*
12186-31	Potassium Acid Phthalate, 300 mg/L COD	236 mL *(8 oz)
22539-31	Potassium Acid Phthalate, 1000 mg/L COD	236 mL (8 oz)

\*Larger sizes available



# OXYGEN DEMAND, CHEMICAL

DR/3000 Procedure Code O.11

Range 0-15,000 mg/L

Reactor Digestion Method\*

## Introduction

The Chemical Oxygen Demand (COD) test is used widely to estimate the amount of organic matter in wastewater. It is a measurement of the oxygen equivalent of the materials present in the wastewater that are subject to oxidation by a strong chemical oxidant, in this case dichromate.

In the reactor digestion method test, the COD procedure is greatly simplified. Small volumes of the water sample are pipetted into vials containing the premeasured reagents, including catalysts and chloride compensator. The vials are digested at 150°C for two hours and cooled; then the COD determination is made either with the spectrophotometer or by titration. This method has been approved for NPDES reporting purposes by the EPA.<sup>†</sup>

Both the titrimetric method and colorimetric method for determining Chemical Oxygen Demand (COD) are contained in this manual. The digestion procedure is needed for both methods, with the choice of the final measurement method left to the analyst. The colorimetric method is the simpler and quicker of the two. The titrimetric method should be used if turbidity or colored species remain after digestion.

### WARNING

*Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings on the labels and read the safety section of this manual. In the procedure, hazardous substances appear in italic typeface wherever they are used in the test and deserve extra care in handling. It is always good practice to wear safety glasses when handling chemicals. Wash thoroughly if contact occurs. Follow instructions carefully.*

## Sampling and Storage

Collect samples in glass bottles if possible. Plastic bottles can be used only if they are known to be free of organic contamination. Biologically active samples should be tested as soon as possible. Samples containing solids should be well-mixed or homogenized to permit the removal of a representative aliquot. NOTE: The following storage instructions are necessary **only** when prompt analysis is impossible. Add 2.0-mL of *Sulfuric Acid*, ACS, for each liter or quart of sample using a glass serological pipet and pipet filler. Mix. Check the sample to be sure the pH is 2 or less. Add more acid if necessary. Samples preserved in this manner can be stored for seven days. Where significant amounts of preservatives are used, a volume correction should be made for the extra acid by dividing the total volume (sample + acid) by the sample volume and multiplying the test result by this factor.

## Digestion

1. Place approximately 100 mL of sample in a clean blender bowl and homogenize at high speed for two minutes. Blending the sample ensures a uniform distribution of suspended solids and thus improves the accuracy and reproducibility of test results. Transfer the homogenized sample to a 250-mL beaker. Place a magnetic stir bar in the beaker and place the beaker on a stir plate. Continuously mix the sample.
2. Turn on the COD Reactor to preheat to 150°C. Wrap a towel around a High Range Plus *COD Digestion Reagent Vial* and cautiously remove the cap. While holding a vial at a 45-degree angle, carefully pipet 0.20 mL of sample into the vial. Use a TenSette Pipet or an equivalent pipetter. For best accuracy, a minimum of three replicates should be analyzed, with the final result being the average value. Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run the COD test with vials that have had reagents spilled from them. See *Notes A and B*.

\*Jirka, A.M., and Carter, M.J., *Anal. Chem.*, 47 (8), 1397 (1975)

<sup>†</sup>Federal Register, April 21, 1980, 45 (78) 26811-26812



## Oxygen Demand, Chemical, Reactor Digestion (continued)

3. Wrap a towel around the vial and cautiously replace the cap. To avoid vapor loss and accidental spillage, be sure caps are well-tightened. Holding the vial by the cap in an empty sink, swirl the vial, using a circular wrist motion, until the contents are mixed.
4. Briefly rinse the COD vial with demineralized water and wipe the vial clean with a paper towel. Place the vial in the preheated COD Reactor. The plastic shield should be in place on the heater block before vials are placed in the reactor.
5. Prepare a reagent blank by repeating Steps 2 through 4, substituting 0.20 mL of distilled or demineralized water in place of the water sample. Use a clean pipet tip for each sample and blank analyzed. Heat the vials for two hours at 150°C. Turn the reactor off and allow the vials to cool to 120°C or less (about 20 minutes). While the vials are still warm, invert each vial several times. Place vials in a cooling rack and allow to cool to room temperature. Measure the COD. One blank from the same lot of vials must be run with each set of samples. The lot number appears to the right of the catalog number in the COD vial container. *See Notes C and D.*

### Procedure

1. To begin the stored program for this test, initiate the program mode by making the following key strokes:

Press:



The STORED PROGRAM light will come on and a wavelength setting of 620.0 for COD testing will appear in the display. The SET WAVELENGTH and ZERO prompting lights will flash.

2. Turn the wavelength selector dial, located on the upper right corner of the instrument, to a setting of 620.

Press:



The SET WAVELENGTH prompting light will turn off and the display will read 0. The ZERO prompting light will continue to flash until the instrument is zeroed in Step 4.

3. Place the COD Vial Adapter in the cell holder of the spectrophotometer with the groove in the adapter facing the front of the instrument. Wipe the vial containing the reagent blank clean with a wet towel followed by a dry one. Insert the vial containing the reagent blank in the adapter with the Hach logo centered on the groove and cover the light shield. *See Note E.*
4. This reagent blank is used to set the zero concentration point. Zero the instrument as follows:

Press:



**Conc**

The ZERO, CONCENTRATION and AUTO UPDATE lights will come on and a concentration value of 0 will appear in the display.

5. Wipe the vial containing the test sample clean with a wet towel followed by a dry one. Place the vial in the adapter, align the Hach logo with the groove, replace the light shield and read the mg/L COD from the display. Multiply the reading by ten to determine the final test result. *See Note F.*



## Oxygen Demand, Chemical, Reactor Digestion (continued)

### Notes

- A. The COD vials contain enough mercuric sulfate to complex more than 20,000 mg/L chloride. As a general rule, accurate results cannot be obtained above this level. For higher chloride concentrations, dilute the sample so that the chloride concentration is less than 10,000 mg/L. If this is not feasible due to low COD values, add 0.50 g *Mercuric Sulfate* to each COD vial prior to addition of sample and blank. Chloride concentrations up to 40,000 mg/L in the 0-15,000 mg/L COD vials cause minimal errors using this modification.
- B. The mixture of potassium dichromate and sulfuric acid is sensitive to ultraviolet light. To avoid reagent deterioration during storage, keep reagent vials protected from all light sources and in a refrigerator if available. Store unused vials in the opaque shipping container. Under normal lighting conditions, the small amount of light that strikes the vials during the digestion period will not affect COD test results.
- C. Many wastewater samples containing easily oxidizable organic materials are digested completely in less than two hours. To use a reduced digestion period, colorimetrically read the sample vials (while still hot) at 15-minute intervals. When the reading remains unchanged, the sample may be considered completely digested.
- D. If a pure green color is obtained in the reacted sample, all the dichromate has been reduced to the chromic state. It will be necessary to repeat the digestion with a diluted sample. For best accuracy, dilute and repeat those determinations that approach 14,000-15,000 mg/L range.
- E. The blank has been found to be stable when kept in the dark. It can be monitored for decomposition by using the %T Mode and reading the color at 620 nm vs. deionized water. Using a vial containing 5 mL of deionized water, press:



A transmittance value of 100.0 will appear on the display. Place the blank in the adapter and record the transmittance value. If the blank transmittance has changed more than 1-2 %T from its initial value, a new blank should be run. A new blank must be run when a different lot of vials is obtained.

- F. If the display flashes 1650 for the 0-15,000 mg/L COD range, the upper limit of the precalibrated range has been exceeded and a sample dilution is needed. Flashing values of -25 indicate the sample has a high negative absorbance value relative to the blank. High negative values indicate a possible contamination of the blank or a procedure error by the user. Small negative values probably are due to small variations between vials or amounts of reagent added and should be treated as a concentration value of zero.

### Precision

In a single laboratory, using a standard solution of 10,000 mg/L COD and two representative lots of reagent, a single operator obtained a standard deviation of  $\pm 100$  mg/L COD for the 0-15,000 mg/L range.

### Accuracy Check

Accuracy of the test can be checked by performing the procedure using 2 mL of 10,000 mg/L COD standard in place of the sample. The test result should be 10,000 mg/L COD. A 10,000-mg/L COD standard can be prepared by drying Potassium Acid Phthalate to a constant weight at 120°C, dissolving 8.500 g in distilled water and diluting to 1 liter. Prepare fresh for each use.



## Oxygen Demand, Chemical, Reactor Digestion (continued)

### Required Reagents and Apparatus

Cat. No.	Description	Unit
272-16	Demineralized Water	946 mL* (qt)
19700-01	TenSette Pipet, 0-1.0 mL	each
16500-10	COD Reactor, 120-240 V	each
44799-00	COD Vial Adapter, DR/3000	each
18641-00	Test Tube Rack (2 recommended)	each
24159-25	<i>COD Digestion Reagent Vials, High Range Plus</i>	25
21856-96	Pipet Tips, for 19700-01	50

### Optional Reagents and Apparatus

500-46	Beaker, 250 mL	each
315-34	Potassium Acid Phthalate	500 g
511-00	Measuring Spoon, 0.1 g	each
979-11	<i>Sulfuric Acid, ACS</i>	473 mL (pt)
1915-20	<i>Mercuric Sulfate</i>	28 g*
45300-01	Stirrer Stand, electromagnetic, 115 V	each
45300-02	Stirrer Stand, electromagnetic, 230 V	each
45315-00	Stir Bar	each
15232-00	Stir Bar Retriever	each

\*Larger sizes available



**Appendix B-13 – Lab Procedures for pH: Method 150.1 or Method 150.2**



## Table of Contents – Appendix B-13

Documents
QC Document – pH – Method 150.1 or Method 150.2
pH Method 150.1 (Electrometric)
EPA Test Method: pH, Continuous Monitoring (Electrometric) – Method 150.2



pH - Method 150.1 or Method 150.2

#### 1.0 Procedure

Perform pH measurements by either Method 150.1 or 150.2 (attached) as appropriate.

#### 2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

#### 3.0 Quality Control Samples

Periodically, reanalyze calibration buffers.



## pH

### Method 150.1 (Electrometric)

STORET NO.

Determined on site 00400

Laboratory 00403

1. Scope and Application
  - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes and acid rain (atmospheric deposition).
2. Summary of Method
  - 2.1 The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode.
3. Sample Handling and Preservation
  - 3.1 Samples should be analyzed as soon as possible preferably in the field at the time of sampling.
  - 3.2 High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis.
4. Interferences
  - 4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity.
  - 4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.
  - 4.3 Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid (1 + 9) may be necessary to remove any remaining film.
  - 4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.
5. Apparatus
  - 5.1 pH Meter-laboratory or field model. A wide variety of instruments are commercially available with various specifications and optional equipment.

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- 5.2 Glass electrode.
- 5.3 Reference electrode—a calomel, silver-silver chloride or other reference electrode of constant potential may be used.  
**NOTE 1:** Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel type filling materials that require minimal maintenance.
- 5.4 Magnetic stirrer and Teflon-coated stirring bar.
- 5.5 Thermometer or temperature sensor for automatic compensation.
- 6. Reagents
  - 6.1 Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is necessary.
    - 6.1.1 Preparation of reference solutions from these salts require some special precautions and handling<sup>(1)</sup> such as low conductivity dilution water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.
  - 6.2 Secondary standard buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, that have been validated by comparison to NBS standards, are recommended for routine use.
- 7. Calibration
  - 7.1 Because of the wide variety of pH meters and accessories, detailed operating procedures cannot be incorporated into this method. Each analyst must be acquainted with the operation of each system and familiar with all instrument functions. Special attention to care of the electrodes is recommended.
  - 7.2 Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart.
    - 7.2.1 Various instrument designs may involve use of a “balance” or “standardize” dial and/or a slope adjustment as outlined in the manufacturer’s instructions. Repeat adjustments on successive portions of the two buffer solutions as outlined in procedure 8.2 until readings are within 0.05 pH units of the buffer solution value.
- 8. Procedure
  - 8.1 Standardize the meter and electrode system as outlined in Section 7.
  - 8.2 Place the sample or buffer solution in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar.
    - 8.2.1 If field measurements are being made the electrodes may be immersed directly in the sample stream to an adequate depth and moved in a manner to insure sufficient sample movement across the electrode sensing element as indicated by drift free (<0.1 pH) readings.
  - 8.3 If the sample temperature differs by more than 2°C from the buffer solution the measured pH values must be corrected. Instruments are equipped with automatic or manual

<sup>(1)</sup>National Bureau of Standards Special Publication 260.



compensators that electronically adjust for temperature differences. Refer to manufacturer's instructions.

8.4 After rinsing and gently wiping the electrodes, if necessary, immerse them into the sample beaker or sample stream and stir at a constant rate to provide homogeneity and suspension of solids. Rate of stirring should minimize the air transfer rate at the air water interface of the sample. Note and record sample pH and temperature. Repeat measurement on successive volumes of sample until values differ by less than 0.1 pH units. Two or three volume changes are usually sufficient.

8.5 For acid rain samples it is most important that the magnetic stirrer is not used. Instead, swirl the sample gently for a few seconds after the introduction of the electrode(s). Allow the electrode(s) to equilibrate. The air-water interface should not be disturbed while measurement is being made. If the sample is not in equilibrium with the atmosphere, pH values will change as the dissolved gases are either absorbed or desorbed. Record sample pH and temperature.

#### 9. Calculation

9.1 pH meters read directly in pH units. Report pH to the nearest 0.1 unit and temperature to the nearest °C.

#### 10. Precision and Accuracy

10.1 Forty-four analysts in twenty laboratories analyzed six synthetic water samples containing exact increments of hydrogen-hydroxyl ions, with the following results:

pH Units	Standard Deviation pH Units	Bias, %	Accuracy as
			Bias, pH Units
3.5	0.10	-0.29	-0.01
3.5	0.11	-0.00	
7.1	0.20	+1.01	+0.07
7.2	0.18	-0.03	-0.002
8.0	0.13	-0.12	-0.01
8.0	0.12	+0.16	+0.01

(FWPCA Method Study 1, Mineral and Physical Analyses)

10.2 In a single laboratory (EMSL), using surface water samples at an average pH of 7.7, the standard deviation was  $\pm 0.1$ .

#### Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 460, (1975).
2. Annual Book of ASTM Standards, Part 31, "Water", Standard D1293-65, p 178 (1976).
3. Peden, M. E. and Skowron, L. M., Ionic Stability of Precipitation Samples, Atmospheric Environment, Vol. 12, pp. 2343-2349, 1978.





## Test Method

# pH, Continuous Monitoring (Electrometric)—Method 150.2

### 1. Scope and Application

1.1 This method is applicable to the continuous pH measurement of drinking, surface, and saline waters, domestic and industrial waste waters.

### 2. Summary of Method

2.1 The pH of a sample is determined electrometrically using a glass electrode with a reference electrode or a single combination electrode.

### 3. Sample Handling and Preservation

3.1 The composition of the water or waste contacting the measuring electrode system must be representative of the total flow from the water body.

### 4. Interferences

4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity.

4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.

4.3 Manually inspect the conditions of the electrodes every 30 days for coating by oily materials or buildup of lime. If oil and grease and/or scale buildup are not present, this time interval may be extended.

4.3.1 Coatings of oil, grease and very fine solids can impair electrode response. These can usually be removed by gentle wiping and

detergent washing. The use of flow-through electrode housings which provide higher flow velocity helps to prevent the coating action.

4.3.2 Heavy particulate matter such as lime accumulation can be removed by careful scrubbing or immersion in dilute (1+9) hydrochloric acid. Continuous monitoring under these conditions benefits from ultrasonic or other in-line continuous cleaning methods.

4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. For best results, meters having automatic temperature compensation should be calibrated with solutions within 5°C of the temperature of the stream to be measured. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.

### 5. Apparatus

5.1 pH Monitor - A wide variety of instruments are commercially available with various specifications and optional equipment. For unattended use, the monitor should be equipped with automatic or fixed



temperature compensation and with a recorder or alarm function.

**5.2** Glass electrode - with shielded cable between electrode and monitor unless preamplification is used.

**5.3** Reference electrode - a reference electrode with a constant potential and with either a visible electrolyte or viscous gel fill.  
**NOTE 1:** Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel-type filling materials that require minimal maintenance.

**5.4** Temperature sensor - for automatic compensator covering general ambient temperature range.

**5.5** Electrode mounting - to hold electrodes; may be flow through (for small flows), pipe mounted or immersion.

## 6. Reagents

**6.1** Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is required.

**6.1.1** Preparation of reference solutions from these salts require some special precautions and handling, such as low conductivity deionized water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.

**6.2** Secondary buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, which have been validated by comparison to NBS standards, is recommended for routine operation. These buffers may be retained for at least six months if kept stoppered.

## 7. Calibration

**7.1** Immersion type electrodes - easily removed from mounting.

**7.1.1** The electrode should be calibrated at a minimum of two points that bracket the expected pH of the water/waste and are approximately three pH units or more apart.

**7.1.2** Repeat calibration adjustments on successive portions of the two buffer solutions until readings are within  $\pm 0.05$  pH units of the buffer value. If calibration problems occur, see 4.3.

**7.1.3** Because of the wide variety of instruments available, no detailed operating instructions are provided. Instead, the analyst should refer to the particular manufacturer's instructions.

**7.1.4** Calibration against two buffers should be carried out at least daily. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data supports a longer period between calibration.

**7.2** Immersion type electrodes - not easily removed from mounting.

**7.2.1** Collect a grab sample of the flowing material from a point as close to the electrode as possible. Measure the pH of this grab sample as quickly as possible with a laboratory - type pH meter. Adjust the calibration control of the continuous monitor to the reading obtained.

**7.2.2** The temperature and condition of the grab sample must remain constant until its pH has been measured by the laboratory pH meter. The temperature of the sample should be measured and the temperature compensator of the laboratory pH meter adjusted.

**7.2.3** The laboratory - type pH meter should be calibrated prior to use against two buffers as outlined in 7.1.

**7.2.4** The continuous pH monitoring system should be initially calibrated against two buffers as outlined in 7.1 before being placed into service. Recalibration (every 30 days) at two points is recommended if at all possible to ensure the measuring electrode is in working order. If this is not possible, the use of electrode testing features for a broken or malfunctioning electrode should be considered when purchasing the equipment.

**7.2.5** The indirect calibration should be carried out at least once a day. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data support a longer period between calibration.

**7.2.6** If the electrode can be removed from the system, but with difficulty, it should be directly calibrated as in 7.1 at least once a month.

**7.3** Flow-through type electrode - easily removed from its mounting.

**7.3.1** Calibrate using buffers as in 7.1. The buffers to be used may be the process stream itself as one buffer, and as a second buffer after adjustment of pH by addition of an acid or base. This will provide the larger volumes necessary to calibrate this type electrode.

**7.3.2** Since the velocity of sample flow-through a flow through electrode can produce an offset error in pH reading, the user must have data on hand to show that the offset is known and compensation has been accomplished.

**7.4** Flow-through type electrode - not easily removed from its mounting.

**7.4.1** Calibrate as in 7.2.

**7.4.2** Quality control data must be on hand to show the user is aware of possible sample flow velocity effects.

## 8. Procedure

**8.1** Calibrate the monitor and electrode system as outlined in Section 7.

**8.2** Follow the manufacturer's recommendation for operation and installation of the system.

**8.3** In wastewaters, the electrode may require periodic cleaning. After manual cleaning, the electrode should be calibrated as in 7.1 or 7.2 before returning to service.

**8.4** The electrode must be placed so that the water or waste flowing past the electrode is representative of the system.

## 9. Calculations

**9.1** pH meters read directly in pH units. Reports pH to the nearest 0.1 unit and temperature to the nearest  $^{\circ}\text{C}$ .

## 10. Precision and Accuracy

**10.1** Because of the wide variability of equipment and conditions and the changeable character of the pH of many process waters and wastes, the precision of this method is probably less than that of Method 150.1; however, a precision of 0.1 pH unit



should be attainable in the range of  
pH 6.0 to 8.0. Accuracy data for  
continuous monitoring equipment  
are not available at this time.

## Bibliography

1. Annual Book of ASTM  
Standards, Part 31, "Water"  
Standard 1293-78, Method D, p. 226  
(1981).



**Appendix B-14 – Lab Procedures for Total Organic Carbon (TOC):  
Method 415.1 with Dohrmann DC-190**



**Table of Contents – Appendix B-14**

<b>Documents</b>
<b>QC Document – Total Organic Carbon – Method 415.1 with Dohrmann DC-190.</b>
<b>Organic Carbon, Total, Method 515.1 (Combustion or Oxidation)</b>



## Total Organic Carbon - Method 415.1 with Dohrmann DC-190

### 1.0 Procedure

Perform Total Organic Carbon analysis in accordance with "Organic Carbon, Total", Method 415.1 (Combustion or Oxidation) and in accordance with chapters 6 and 10 of the operating manual for the Dohrmann DC-190 high temperature organic carbon analyzer as attached.

### 2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

### 3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.



# ORGANIC CARBON, TOTAL

## Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680

Dissolved 00681

### 1. Scope and Application

1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.

1.2 The method is most applicable to measurement of organic carbon above 1 mg/l.

### 2. Summary of Method

2.1 Organic carbon in a sample is converted to carbon dioxide ( $\text{CO}_2$ ) by catalytic combustion or wet chemical oxidation. The  $\text{CO}_2$  formed can be measured directly by an infrared detector or converted to methane ( $\text{CH}_4$ ) and measured by a flame ionization detector. The amount of  $\text{CO}_2$  or  $\text{CH}_4$  is directly proportional to the concentration of carbonaceous material in the sample.

### 3. Definitions

3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:

- A) soluble, nonvolatile organic carbon; for instance, natural sugars.
- B) soluble, volatile organic carbon; for instance, mercaptans.
- C) insoluble, partially volatile carbon; for instance, oils.
- D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
- E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.

3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

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4. Sample Handling and Preservation
  - 4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples. **NOTE 1:** A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.
  - 4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.
  - 4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified ( $\text{pH} \leq 2$ ) with HCl or  $\text{H}_2\text{SO}_4$ .
5. Interferences
  - 5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.
  - 5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.
6. Apparatus
  - 6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.
  - 6.2 Apparatus for total and dissolved organic carbon:
    - 6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.
    - 6.2.2 No specific analyzer is recommended as superior.
7. Reagents
  - 7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.
  - 7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.  
**NOTE 2:** Sodium oxalate and acetic acid are not recommended as stock solutions.
  - 7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.
  - 7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.



- 7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

**NOTE 3:** This standard is not required by some instruments.

- 7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

- 8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

- 8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

- 9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Bias, %	Accuracy as Bias, mg/liter
4.9	3.93	+ 15.27	+ 0.75
107	8.32	+ 1.01	+ 1.08

(FWPCA Method Study 3, Demand Analyses)

**Bibliography**

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 2574-79, p 469 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 532, Method 505, (1975).



## SECTION 6

# OPERATION

---

### INTRODUCTION

This section contains instructions for routine operation along with detailed descriptions on how to operate and calibrate the different modes.

### 6.1 ROUTINE OPERATION

SUMMARY
<ul style="list-style-type: none"><li>* Daily Start-Up</li><li>* Daily Operation</li><li>* Daily and Long-term Shutdown</li></ul>



## DAILY START-UP

### Check utility supply.

Enough carrier gas for a day's operation.

Acid reservoir at least 1/3 full.

### Replenish IC chamber.

Confirm the IC chamber is half full (gas off).

Fill the IC chamber by using the "Acid to IC chamber" function (press **MAIN 2 5**). Each use of this function will result in 20 pulses and is equivalent to 2 ml of acid.

### Turn on gas.

Press **CARRIER**.

For Boat Users :

Connect the 1/8 inch PTFE line from the boat module furnace to the DC-190 dehumidifier (see Figure 4.8).

### Check system status.

(Press **MAIN 1** to view the status menu.)

Flow rate = 180 - 220 cc/min.

Dryer temperature = 0 - 10 °C

Furnace Temperature = Furnace set point  
(Furnace light is green.) For most applications, the temperature should be 680°C.

Confirm or change set-up number on display  
(see Section 6.8).

### Check set-up.

(See Section 6.2 for help in choosing set-up.)

Modes last used are lit up. Make any changes for the day and print the set-up parameters. System is ready for analysis.



## DAILY OPERATION

**Press START when ready.**

It is good practice to run a check standard at the beginning of the day before analyzing unknowns, especially if any conditions have been changed. Update calibration if needed. See Section 6.3 for notes on operating and calibrating.

## DAILY SHUTDOWN

**Check the RUN status.**

The unit should not be in a RUN mode.

**For Boat Users :**

Disconnect the 1/8 inch PTFE line which runs from the boat furnace to the dehumidifier.

**Shut off the gas.**

Press **CARRIER** .

**NOTE:** The furnace and the NDIR should be left on unless the unit is going to be relocated or will not be used for a long time. Frequently turning the furnace on/off reduces the life of the heater element. The NDIR requires at least 2 hours for stabilization after power up.



## 6.2 SELECTING THE ANALYSIS PARAMETERS

Most analysis have three parameters:

- 1) Analysis mode.
- 2) Inlet mode.
- 3) Volume.

**NOTE:** The ASM and RSM operating modes have other parameters which must be selected. See Sections 6.4 and 6.5 for guidelines in selecting these parameters.

### SELECT THE ANALYSIS MODE

Use Table 6.1 to match your application to an analysis mode. The default mode is **NPOC**. To set another mode, press the corresponding button.

**Table 6.1**  
**ANALYSIS MODE SELECTION**

ANALYSIS MODE	APPLICATION	METHOD
NPOC	Any water sample.	IC purged from sample at sparging station. Inject into TC port. $\begin{array}{c} \text{TC} \\ \text{NPOC} \text{ -----} > \text{CO}_2 \\ \text{Furnace} \end{array}$
TOC	Any water sample. Method of choice when sample has no volatiles.	TOC = TC - IC Two (2) injections per analysis. DC-190 calculates the difference. See See TC and IC descriptions.
IC	Any sample where dissolved CO <sub>2</sub> or carbonate concentration is of interest.	Sample injected into IC port. $\begin{array}{c} \text{IC} \\ \text{IC} \text{ -----} > \text{CO}_2 \\ \text{Chamber} \end{array}$
TC	Any water sample.	Sample injected into TC port. $\begin{array}{c} \text{TC} \\ \text{TC} \text{ -----} > \text{CO}_2 \\ \text{Furnace} \end{array}$
POC	Water sampler where volatile organics or other purgeables are of interest.	Sample is sparged at POC sparge station. LiOH scrubber removes IC from sparged gas. $\begin{array}{c} \text{TC} \\ \text{POC Gas} \text{ --} > \text{IC Scrubber} \text{ --} > \text{POC} \text{ -----} > \text{CO}_2 \\ \text{Furnace} \end{array}$
Boat Option, TC	Solids, sludges, slurries and waters with particulates greater than 0.5 mm.	Sample introduced onto platinum boat. Boat pushed into 183 furnace. 800°C $\begin{array}{c} \text{Sample} \text{ -----} > \text{CO}_2 \end{array}$



## SELECT THE INLET MODE

The default inlet mode is **SYRINGE** . To select a different mode, refer to the following Table, then press the button corresponding to the new inlet mode.

**Table 6.2**  
**INLET MODE SELECTION**

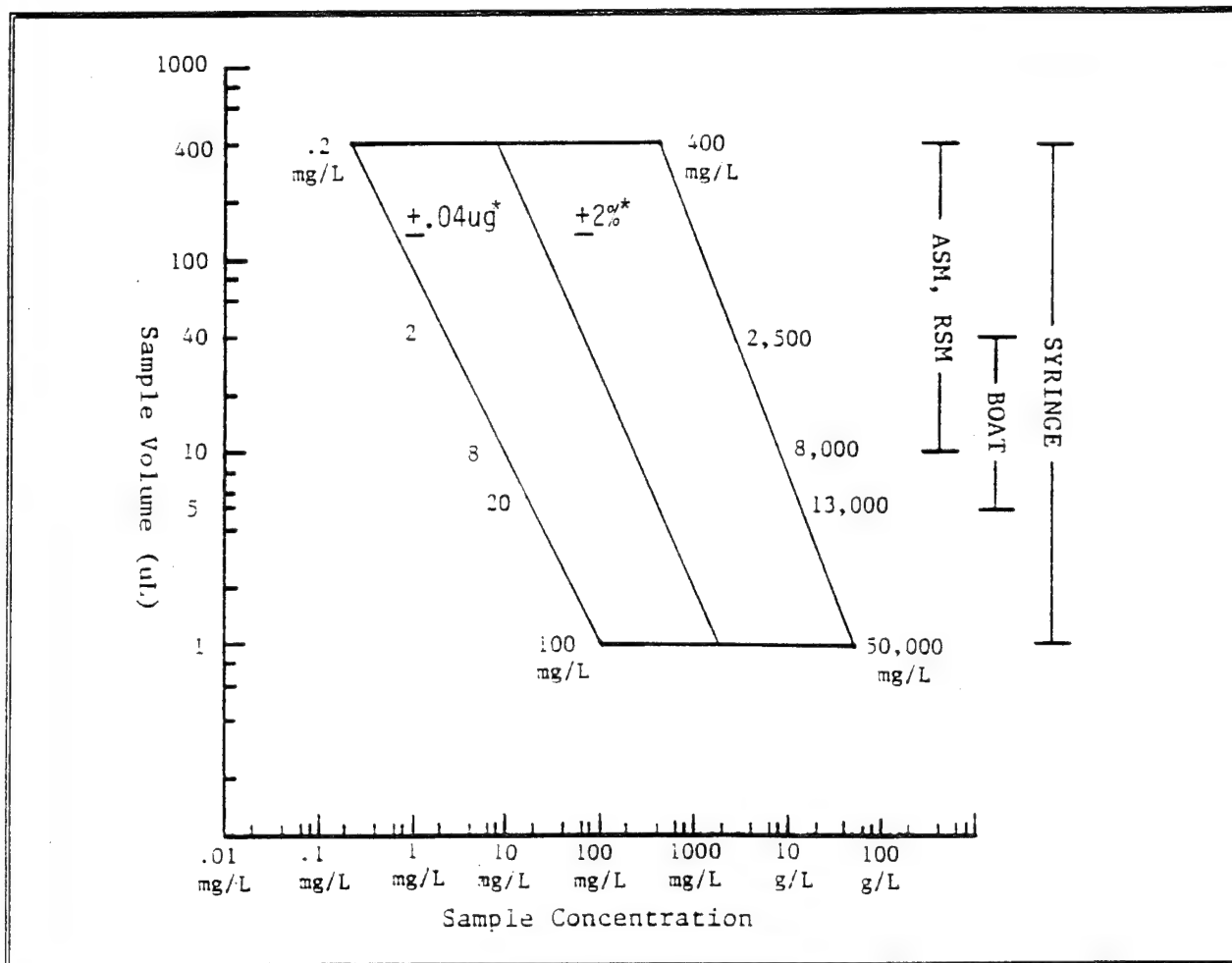
ANALYSIS MODE	INLET MODE	DEFAULT		POSSIBLE VOLUME (ul)
		Volume (ul)	Range (mgC/L)	
NPOC TOC IC TC	Syringe	50	1 - 2000	1 - 400  20 - 200 *
	ASM	50	1 - 2000	10 - 400
TOC IC TC	RSM	50	1 - 2000	10 - 400
TC NPOC	Boat	40	2 - 4000	5 - 40
POC	N/A	10 mL	.01 - 20	2 - 10 mL

\* This is the range for the manual micropipettor which is used with the **SYRINGE** mode.



## SELECT VOLUME

The default volume and corresponding concentration range for each inlet mode are shown in the previous table. If the default concentration range is unsuitable, a better sample volume may be selected using Figure 6.1. Enter the new sample volume on the inlet mode menu.



\* Expected precision. See Section 1.4.

FIGURE 6.1 Concentration Range vs. Sample Volume

### EXAMPLE:

Expected sample concentration range = 5 to 5,000 ppm.

From Figure 6.1, 20 ul gives 4 to 6,000 ppm.

(Note the logarithmic scales.)

20 ul is compatible with all inlet modes, except POC.



## 6.3 MANUAL OPERATION

Use these instructions for syringe or micropipettor operation in NPOC, TOC, IC, TC or POC modes. The following table shows the general operation sequence for syringe injections. Specific notes for each analysis mode follow the table.

### GENERAL OPERATION SEQUENCE - ALL MODES

- \* If it is desired to save the current operating parameters before making any changes, select a new set-up number (see Section 6.7).
- \* Choose set-up.
- \* Have the syringe filled and ready. (Have the septum installed as shown in Figure 6.2.)
- \* Press **START** .
- \* Inject the sample. (Review the injection technique for the mode selected.)
- \* At the conclusion of the analysis, the screen will display the final ppm value along with:

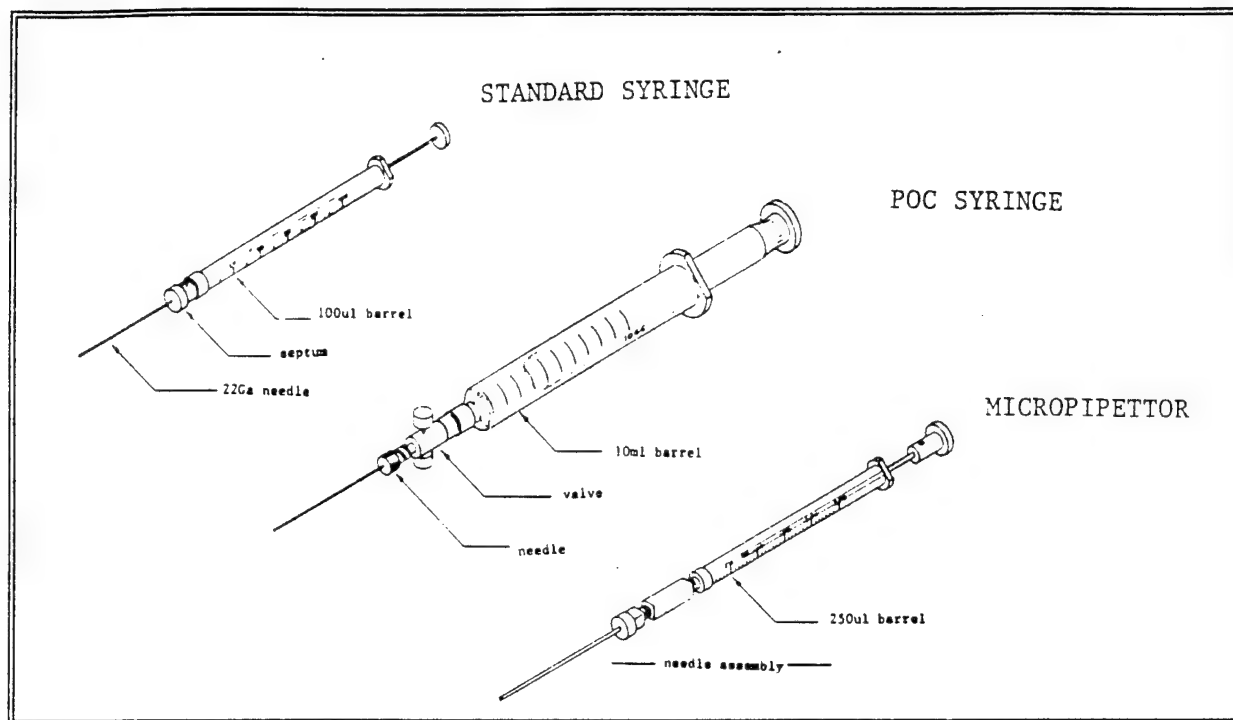
**Continue Y/N ?**

(This question must be answered before the system will perform any other action.)

- \* Press **YES** to make more injections.
- \* Press **NO** to end the run.
- \* Press **STOP** to end the run after the current analysis. To terminate the run, immediately press **STOP** five times.



## ABOUT SYRINGES



**FIGURE 6.2. SYRINGE ASSEMBLIES**

Assemble the syringes and micropipettor as shown in Figure 6.2. Always have a grey septum attached to the syringe or pipettor.

It is important for reliable sample introduction to use blunt-point needles such as those supplied with the DC-190. Side-port needles should not be used except on the POC syringe.

The 100 uL syringe (P/N 060-871) provided with the DC-190 has a 22S gauge (0.006 inch I.D.) needle. The 22 gauge (0.016 inch I.D.) replacement needles (P/N 060-872) are provided in the DC-190 operating kit for sample types requiring a larger I.D. needle.

Also available are a micropipettor barrel (250 uL syringe barrel, P/N 060-875) and a micropipettor needle (P/N 888-297). The micropipettor is used for samples containing particulates up to 0.5 mm diameter or samples which are incompatible with (react with or corrode) a stainless steel needle. The micropipettor probe should be used with a 250 uL syringe barrel only.



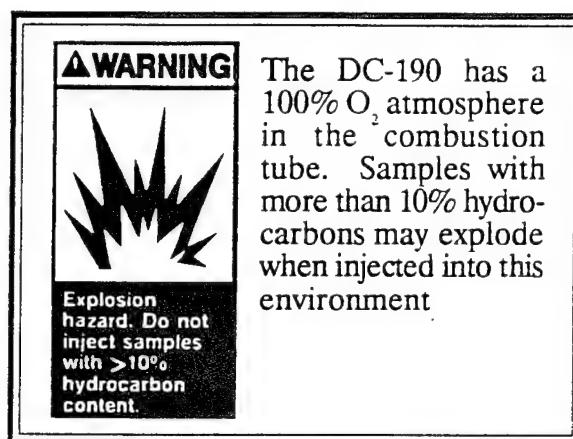
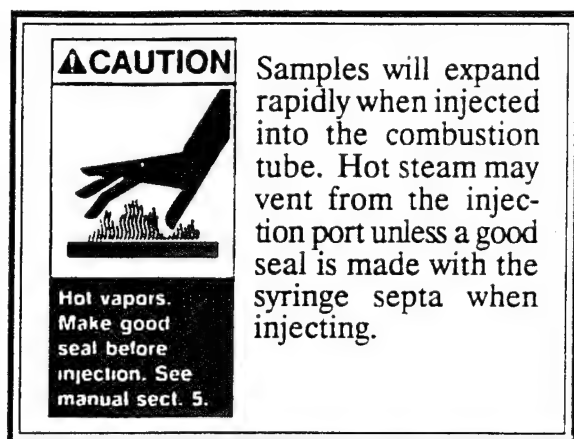
## TC or IC

### Injection Technique

As soon as the INJECT light comes on, press **OPEN/CLOSE**

Immediately insert the syringe into the injection port that has the illuminated LED.

Make seal during injection by pressing the grey septum against the port.



Inject at 50 ul/sec rate.

Withdraw the syringe and immediately press **OPEN/CLOSE** to close the port.

For 1 - 10 ul volumes, wait 5 seconds in between injecting and withdrawing syringe.

### Micropipettor Users:

When using a micropipettor, wipe off the outside of the probe after drawing up the sample.

For volumes below 50 uL, the injection rate is crucial to obtaining reproducible results. Make the injection rapidly without jarring the syringe. (**HINT:** After withdrawing the syringe, look at the tip. If it is wet on the outside, inject faster; if it is partially empty, inject slower.)

Wait 10 seconds after injecting before withdrawing the pipettor for all volumes.

### Sample Pretreatment

None, unless the samples are inhomogeneous or contain large particulates (> 0.5 mm diameter).



## TOC

(This is a combination of the TC and IC modes.)


### Injection Technique


Use the same technique as for the TC and IC modes.

Make two injections per analysis.

The first injection goes in the TC port.

Have the syringe filled and ready for the second injection which is made to the IC port. Look for the prompt from the display.

	<b>CAUTION</b> Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.
<b>Hot vapors. Make good seal before injection. See manual sect. 5.</b>	

	<b>WARNING</b> The DC-190 has a 100% O <sub>2</sub> atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.
<b>Explosion hazard. Do not inject samples with &gt;10% hydrocarbon content.</b>	

**NOTE:** When high pH samples are expected, treat combustion tube with 2 injections of 100 ul of pH1 HCl or HNO<sub>3</sub> solution.



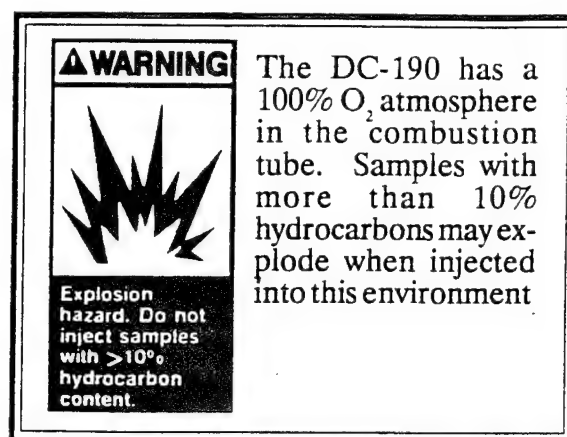
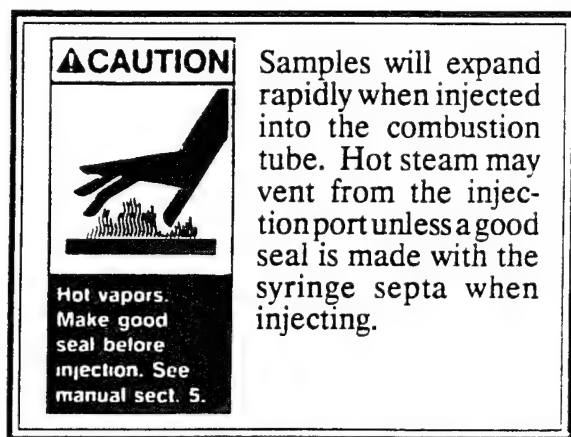
## NPOC

(This is the default analysis mode.)

### Injection Technique

Use the same technique as for the TC and IC modes.

Inject into the TC port only.



### Sample Pretreatment

The sample must be sparged prior to injection to remove the IC.

#### To sparge the sample:

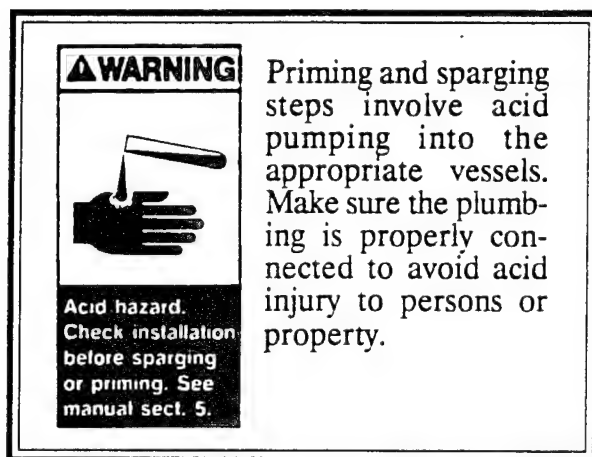
- Pour about 10 mL of sample into a 20 mL vial (P/N 889-726).
- Screw the vial into Sparger A or Sparger B.
- Press **A** or **B**, and then **1** to start sparging.
- The sample will be automatically acidified. Each unit of "Add acid" is equivalent to 100 ul.
- Sparging will stop automatically at the end of sparge time.
- Remove the vial and cap it until the analysis is run.

Two samples can be sparged simultaneously.

Samples containing large particulates (> 0.5 mm) must be pretreated as directed in Section 10.2.



## POC



### Injection Technique

As soon as the INJECT light comes on, inject the sample into the POC sparger through the injection port.

When the analysis is over, withdraw the remaining sample from the sparger with the syringe.

### Sample Pretreatment

None.

### How to Fill the Syringe

Remove the plunger from the syringe and close the syringe valve and needle. Open the sample or standard container, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 10 ml.

This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample container, the analyst should fill a second syringe at this time in case the first analysis is unsuccessful.



## 6.4 AUTOSAMPLER OPERATION

### INTRODUCTION

The DC-190 Autosampler (ASM) option is designed for unattended operation for many hours. The sample tray holds 32 8 mL vials. Automatic acid addition and sparging are provided by the sparge tower to remove inorganic carbon for NPOC analysis. The sample probe may be rinsed with either water and/or sample between analyses. The ASM can handle samples with particulates up to 0.5 mm and the sample may be stirred with gas before the sample is drawn to insure uniform sampling. Cross-contamination is minimized by the use of non-wetting materials for all sample contacting parts. Sample vials may be marked as blanks or standards for automatic calibration of the system during the ASM run.

The ASM offers an autoranging capability which will adjust the sample volume to maintain the peak integral within the range of the detector. Since the dynamic range of the DC-190 system is very wide (10,000 to 1), activation of the autoranging will normally be a very rare event. When this feature is active, the DC-190 will check the first replicate of a vial in the ASM mode to verify that the peak integral is within range. If the peak integral is below range, the result will be printed, but ignored in future statistical calculations. The injection will then be repeated, but with a volume 5 times larger than the original injection. If the peak integral is over range, a similar procedure is followed with a volume one fifth the original volume. The volume adjustment will be repeated until the peak integral is within range. If an adjustment would result in a volume outside the 10 to 400 uL range, the volume will be set to either 10 or 400 uL as appropriate and no further adjustment will be made. The original injection volume will be restored at the beginning of the next sample vial. The accuracy of the autoranged data may suffer somewhat because the ASM was not calibrated with the new volume. The inaccuracy without autoranging is potentially much worse, however, than with autoranging. If desired, the results of autoranged data may be rechecked later.



Below is a table of expected and observed volumes for the ASM. These values are approximate and will vary from instrument to instrument. This volume variation only affects autoranged data. This will not apply to normal calibrated ASM data because the same volume is used for analysis.

VOLUME (uL)	
<u>Expected</u>	<u>Observed</u>
10	10.3
20	19.5
40	35.4
80	70
100	92
200	194
400	400



## OPERATION

- \* Refer to DAILY START-UP in Section 6.1 to prepare the analyzer for operation.
- \* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).
- \* Refer to Section 6.2 and select the analysis mode and volume desired. See Table 6.3 for guidelines to set the other operating parameters.
- \* Place the vials in the sample tray beginning with tray position 1. Refer to Table 6.4 and mark the vials as blanks, standards, or samples as appropriate. Mark the first empty tray position after the samples as indicated in Table 6.4 to terminate the run.
- \* Clean and fill the rinse bottle with DI water if water rinses were called for on the Rinse\stir menu.
- \* Check that the acid bottle is at least 1/3 full of acid solution if set up for NPOC analysis.
- \* Check that the printer is ready and has sufficient paper.
- \* Press **START**.
- \* There are two ways to end the run before completion. Press **STOP** to end the run after the current analysis. To terminate the run immediately, press **STOP** five times. After an immediate bail out, the ASM may have to be returned to its resting position. The sparge arm may be raised by selecting "Raise sparge arm" ( 1 ) on the "Sparge arm menu" ( **MAIN 2 5 3 3** ). The sample arm may be returned to the rinse bottle position by selecting "Move arm to rinse" ( 4 ) on the "Sample arm" menu ( **MAIN 2 5 3 2** ). Always check the "Furnace/IC ports" menu ( **MAIN 2 5 5** ) to be sure the inlet ports are shut (even if the indicator lights next to the ports are not lit).



**TABLE 6.3**  
**ASM OPERATION PARAMETER GUIDELINES**

<b># of repeats</b>	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats, with 3 being the default.
<b>Sparge time (min)</b>	The default time (3 minutes) should be satisfactory for almost all samples as long as the pH is in the proper range (see "Acid volume" below). This option is applicable to the NPOC mode only.
<b>Acid volume</b>	The pH must be adjusted to a value less than 4. It may be necessary to check a few samples after acid addition and make adjustments by trial and error until the acid addition matches the particular samples being analyzed. The default is 1 (each unit of acid volume is equivalent to 100 ul). This option is applicable to the NPOC mode only.

The following selections are on the "Rinse and /or stir" menu:

<b># of rinses w/water</b>	This option specifies the number of times the ASM sample probe and loop will be rinsed with water between each vial.
<b># of rinses w/sample</b>	Similar to the above option except that the ASM will rinse with sample before the first injection from each vial.
<b>Sample stir time (sec)</b>	Specifies the time that the sample will be stirred before the sample is drawn into the sample loop. The allowed range is 0 - 30 seconds (default = 0). In most applications, 15 seconds will provide effective stirring. Stirring is accomplished by bubbling gas out of the sample probe to suspend particulates and obtain a more uniform sample.
<b>Auto-range</b>	When set to "Yes", the DC-190 will automatically adjust the injection volume. "No" is the default setting. See the INTRODUCTION to this section for details on this feature.
<b>CG off after</b>	The default "No" means the carrier gas (CG) will not be turned off at the end of an ASM run. A "Yes" will cause the carrier gas to be turned off 10 minutes after the end of an ASM run. During this period, the red light in the <b>START/STOP</b> button will blink as if the run is still in progress.



**TABLE 6.4**  
**ASM VIAL MARKERS**

VIAL	PEG POSITION		INDICATION
	INNER*	OUTER**	
No	No	No	Skip Position
Yes	No	No	Sample
Yes	Yes	No	Blank
Yes	No	Yes	Standard
Yes	Yes	Yes	Rinse Sample***
No	No	Yes	Terminate Run

\* Peg hole closer to center of sample tray.

\*\* Peg hole closer to sample vial.

\*\*\* Sample is used for rinse only (no analysis).

**NOTE:** If the printer runs out of paper or jams during a run "Print last run" ( **MAIN 2 3** ) will reprint the run data from a buffer. This allows data otherwise lost to be retrieved. The buffer which retains the data is not large enough, however, to hold a complete run of data in all cases. This buffer has sufficient capacity to hold data from approximately 32 vials with 3 replicates per vial in modes where each replicate requires one line to print (TC, IC, or NPOC). In the TOC mode, each replicate requires three lines to print. In this mode, the buffer will only hold approximately 10 vials with 3 replicates per vial. The buffer is filled on a first in first out basis so that the data remaining at the end of the run will be the last data point back until the buffer is full.



## 6.5 OPERATION OF THE RSM OPTION

The RSM option allows the continuous sampling of a sample stream which is tapped to flow through the RSM sample cell. The ASM will perform the designated number of replicates on the sample stream and then wait for a designated time period. The sampling cycle is then repeated. The TC, IC, and TOC analysis modes may be performed using the RSM option. However, if the sample stream IC and TC levels are not constant, the accuracy of the TOC analysis may suffer due to the time lag between the IC and TC portions of the analysis.

- \* Adjust the sample flow rate to the sample cell by slowly opening the needle valve (counter clockwise) until the water level stabilizes slightly above the drain port of the sample cell.
- \* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).
- \* Select **TC**, **IC**, or **TOC** (see Section 6.2 for selection guidelines) and then **RSM** to set the analysis mode. Verify that the operating parameters are set to the desired values. Use the guidelines in Table 6.5.
- \* Calibrate the DC-190 according to the RSM calibration procedure in Section 6.8.
- \* Press **START** to begin the analysis. The RSM will continue until manually stopped.
- \* To stop the analysis, press **STOP** (same button as **START**). This will stop the DC-190 at the end of an analysis in progress or immediately during the time between runs. To stop the run immediately during an analysis, press the **STOP** button 5 times.



**TABLE 6.5**  
**RSM OPERATING PARAMETER GUIDELINES**

Sample volume	See Figure 6.1
# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats with 3 being the default.
Time between runs	This is the time from the conclusion of the last replicate of a group to the beginning of the first replicate of the next group. The allowable range is 0 to 54 minutes with a default of 0 minutes.



## 6.6 OPERATION OF THE BOAT OPTION

Use the boat sampler for slurries, sludges, solids, and suspensions. Operate in either the **TC** or **NPOC** mode. Refer to "Installation and Operation of the 183 Boat Sampling Module" (P/N 915-240) for sample introduction instructions (Section V, Parts 5A and 5B). The DC-190 calculates ppmC from liquids or solids.

SAMPLE TYPE	SAMPLE INTRODUCTION	CONCENTRATION UNITS
Liquids, light slurries, suspensions	See 183 Instructions for <b>Liquids</b>	mg/L
Solids, heavy slurries	See 183 Instructions for <b>Solids</b>	ug/g

- \* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).
- \* Press **BOAT TC** or **NPOC**.
- \* Press **1** until the appropriate units are displayed.
- \* Introduce the sample into the boat - see "Installation and Operation of the 183 Boat Sampling Module".
- \* Press **START** and follow the 183 instructions.
- \* If ug/g units are selected, enter the sample weight when asked - "Sample weight (mg)?".
- \* **SOLIDS ONLY:** Enter the sample weight when asked - "Sample weight (mg)?".



## 6.7 CALIBRATION

The DC-190 offers a choice of either one point or two point calibration. Two point calibration is equivalent to subtracting the blank value automatically. The DC-190 system always calculates a two point linear calibration. If only a single point calibration is desired, the System Blank may be set to 0 before updating the Calibration Factor. In this case the System Blank will remain 0 after updating the Calibration Factor resulting in a single point calibration. Since the system blank for IC is normally insignificant, its value is set to zero and IC analysis always has one point calibration. When two-point calibration is used, both calibration factor and system blank are recalculated each time either the calibration factor or system blank is updated. In TOC mode, the system uses TC value for calibration and blank update.

The DC-190 system provides a common calibration set (calibration factor and system blank) for SYRINGE, ASM, and RSM modes. POC and BOAT modes have their own calibration sets. When changing inlet mode from SYRINGE to ASM or RSM, calibration stays the same. When changing inlet mode from SYRINGE, ASM, or RSM to POC or Boat, calibration changes accordingly. The multiple set-up function (see Section 6.8) provides capability to store and retrieve up to 5 calibration sets.

Since SYRINGE and ASM/RSM calibrations are not necessarily the same, calibration for these modes should be done separately. Use the multiple set-up function to store the different calibration sets.

SUMMARY
<ol style="list-style-type: none"><li>1. System Blank</li><li>2. Calibrating Syringe, POC, or Boat Modes</li><li>3. Calibrating The ASM Mode</li><li>4. Calibrating The RSM Option</li><li>5. Omitting Outlier Data</li><li>6. Calibration Equations</li></ol>



## SYSTEM BLANK

System blank is defined as the response contributed by the analyzer when carbon-free water sample is injected and analyzed. In reality, it is very difficult to produce and preserve the carbon-free water. Thus the true system blank and the carbon content of the water sample cannot be accurately distinguished. However, the carbon content of high purity water can be below the detection limit (.2ppmC) and the response with such water may be assumed as the system blank. When it exists, the blank value is subtracted from every analysis except in IC mode where blank is always assumed to be zero.

The system blank becomes increasingly important for analyses below 10 mgC/L as shown :

MODE	VOLUME	TYPICAL BLANK (mgC/L)
TC    NPOC	400 ul	.10    -    .40
IC	400ul	0*
POC	10ml	0    -    .03
BOAT	40ul	2.0    -    4.0

### Factors affecting the blank :

- Cleanliness of syringes, spargers and IC chamber.
- Sample handling.
- Age and sample history of TC and boat combustion tubes.
- Dehumidifier temperature.



## CALIBRATING THE SYRINGE , POC , or BOAT INLET MODES

See "SYSTEM BLANK" earlier in this section for guidelines to determine whether a two point calibration is needed for the samples to be analyzed.

- \* Analyze a standard in the analysis mode to be used. An average of at least two determinations is recommended. Respond **NO** to the prompt "Continue yes/no?" when satisfied with the results.
- \* Outlier data can be omitted at this point if desired. See the section "OMITTING OUTLIER DATA" at the end of this section for details on how to do this.
- \* Press **CALIBRATE** to review the calibration menu :

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

- \* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.
- \* Press **5** to update the Calibration Factor. The new calibration factor will be calculated and displayed on the menu.
- \* To complete a two point calibration, if desired, repeat the above procedure with a blank sample. Use the cleanest reagent water available (less than 0.150 mgC/L). Press **6** to update the System Blank.
- \* The DC-190 is now calibrated for the selected analysis mode.



Analyze a check standard with each sample set. If the reported value deviates from the expected value by more than 2%, re-calibrate the system.

**Note To Boat Users:**

It is easy to use a liquid standard to calibrate the DC-190 even when using "ug/g" units to analyze solid samples. For example, to obtain 10 mg of sample, simply inject 10 ul of standard. This relationship holds as long as the density of the standard is 1 g/mL, which will be true for most water-based standards.



## CALIBRATING THE ASM INLET MODE

- \* Select the ASM operating parameters as described in Section 6.4 and press **START** to begin analyzing the standard.
- \* Place the vials of standard in the first tray positions. It is recommended that two vials of standard be placed next to each other at the beginning of the ASM sample tray. Place a peg in the outer hole next to the second vial to mark it as a standard for calibration (see Table 6.4).
- \* If blanks are to be determined, place two or three vials of blank immediately following the vials of standard. In most circumstances, two vials are sufficient. For best accuracy at low levels, three vials are recommended. Place a peg in the inner hole next to the last of the two or three blank vials to instruct the DC-190 to determine a new blank value (see Table 6.4).
- \* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

- \* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.
- \* Place the sample vials in the sample tray following the standard and blank vials, and run the analysis according to the operation instructions in Section 6.4. The DC-190 will automatically calculate and use the calibration factor and blank value.



## CALIBRATING THE RSM OPTION

The RSM mode is easiest to calibrate using a vial of the desired standard rather than by pumping the standard through the RSM sample cell. This method is described in the following steps:

- \* Lift the sample cell from its holder and secure it in the clip located to the left of the black cell holder.
- \* Place an ASM vial (P/N 080-140) containing the standard solution into the black cell holder.
- \* Select the RSM operating parameters as described in Section 6.5 and press **START** to begin analyzing the standard.
- \* Since the RSM does not stop automatically, it is necessary to manually stop it by pressing **STOP** (the same button as **START**) during the last desired replicate of the standard. The DC-190 will then stop at the end of the current analysis.
- \* Outlier data can be omitted at this point if desired. See the Section "OMITTING OUTLIER DATA" at the end of this Section for details on how to do this.
- \* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

- \* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If subtraction of the blank is not desired, make sure the System Blank is set to 0. Make any necessary changes.
- \* Press **5** "Update cal factor" to calculate and store a new calibration factor.
- \* Repeat the above procedure with a blank sample and press **6** "Update system blank" on the "Calibration" menu if an update of the system blank is desired.



## OMITTING OUTLIER DATA

The DC-190 provides the ability to reject outlier data when operated in the manual modes (Syringe, Boat, and POC) and the RSM mode (no provision for outlier rejection is made in the ASM mode). A new average and standard deviation are calculated after the data is rejected. This feature saves having to re-run a data set due to a bad data point when updating the Calibration Factor or System Blank. The DC-190 will not allow the number of replicates to be reduced to less than 2 as a result of data rejection. Data rejection is accomplished by the following steps:

- \* Complete the run by responding **NO** in one of the manual modes or **STOP** in the RSM mode (see the calibration instructions for the mode in use) to the prompt at the end of the analysis. Three or more replicates must have been generated.
- \* Select the "Auxiliary functions" menu ( **MAIN 2** ) and press **1** "Omit an outlier".
- \* At the prompt, enter the number of replicates to reject. Each replicate to be rejected will be prompted for separately. Enter a replicate number after each prompt.
- \* New statistics will be displayed on the screen and printer. An update of the Calibration Factor or System Blank will now be based on the new average value.
- \* If the "Omit an outlier" menu item is selected again after the current data set has been edited, the DC-190 will start the data rejection over and ignore the previous data editing.



## CALIBRATION EQUATIONS

The following equations are used in the DC-190 system.

The equation for determining a calibrated result is:

$$y = (Fx - b) / V$$

- where:
- y = Concentration (calibrated) of sample.
  - x = NDIR peak with background subtracted. Normally invisible to the user. The displayed value, y, may be made to equal x by setting F, b, and V to the appropriate values (1, 0, and 1, respectively)
  - F = "Calibration Factor". This is the slope of the linear fit line.
  - b = Intercept. This is an internal parameter which is invisible to the user.
  - SB = "System Blank" = b/V.
  - V = Sample volume (or mass).

The quantities F and SB are the ones displayed on the calibration menu and are the ones which can be edited directly.

The Calibration Factor and Blank are calculated by:

$$F_n = F_o (C_s / y_s)$$

$$b_n = b_o (F_n / F_o)$$

- where:
- C<sub>s</sub> = Concentration of the standard.
  - o = Old value.
  - n = New value.
  - s = Value for Standard.



These are the equations used internally by the DC-190 system. Both  $F_n$  and  $b_n$  are recalculated each time either the Calibration Factor or the System Blank is updated. It should be noted that if the old value  $b_o$  is already 0, the new value  $b_n$  and therefore SB will also be 0. This provides a means to have the system effectively do a one point calibration update when it calculates a new Calibration Factor. These equations may also be used to manually calculate the values and enter them on the "Calibration" menu directly.



## 6.8 USING THE MULTIPLE PARAMETER SETS

The DC-190 provides the capability to store 5 complete sets of operating parameters. This capability allows the user to return to a previously defined set of operating parameters without having to re-enter the parameters. The parameter set includes the inlet mode, the analysis mode, the parameters appropriate to the analysis/inlet mode as well as the Calibration Factor and System Blank..

One of the parameter sets is always the "working" set-up. This is the parameter set associated with the current set-up number. Any run started will now use the parameter values currently contained in the working parameter set. As changes are made to the operating parameters, these changes are made to the working set-up.

When a new set-up number is selected, the parameter values in the previous set-up are saved as they were at the time of the new selection. The working parameter set now takes the values associated with the new set-up number. Any run started will use the new parameter values and any parameter changes are now made to the new parameter set.

Returning to the previous set-up number will restore the operating parameters to the state they were in when the set-up number was last used.

If it is desired to save the current set of parameter values for future re-use, a new set-up number should be selected before starting to define a new parameter set.

To determine the set-up number:	Display the "System status" menu ( <b>MAIN 1</b> ) . Line 5 "Analysis set-up" indicates the current Set-up number.
---------------------------------	---

To change to another set-up number:	Select the "System status" menu ( <b>MAIN 1</b> ) and then "Analysis set-up" ( <b>5</b> ) and enter the new Set-up number. This saves the current parameter set.
-------------------------------------	--



To print the current parameter set:

Press the analysis mode button with the lit LED and then select the "Print set-up" option on the displayed operating parameter menu.

To print all the parameter sets:

Display the "System status" menu ( **MAIN 1** ).  
Press **6** "Print set-up selections".



## USING THE CLIPBOARD

A clipboard is provided in the DC-190 system which allows the Calibration Factor and System Blank to be copied from one parameter set to another. This feature can save time and effort when changing from parameter set to another after the system has been calibrated. Use the following steps:

- \* Select the "Other actions" section of the "Calibration" menu ( **CALIBRATE 7** ).
- \* Verify that the "Analysis set-up" shown on line 4 is the one from which to copy the calibration factors. If not, select **4** "Analysis set-up" and enter the desired set-up number.
- \* Select **2** to save the calibration factors.
- \* Enter the number of the new parameter set on line 4 and select **3** to copy the calibration factors.

The new parameter set now contains the same Calibration Factor and System Blank as the one copied.



# DC-190 Operation Guide

## DAILY START-UP

## DAILY SHUT-DOWN

## OPER. & CAL

## MAINTENANCE

## DO'S & DON'T'S

1. Gas @ 30 Psi.
2. Check that the acid bottle is 1/3 full.

3. Confirm that the IC chamber is 1/2 full (gas off)
4. Fill IC chamber by using the prime acid function.

5. Press **CARRIER**. Check that gas is flowing in IC chamber.

6. Ensure there is water in the dehumidifier.

7. Observe green lights on carrier & furnace.

8. Check for: flow rate 180-220cc/min, dehumidifier temp. 0-10°C, and furnace temp. 680°C. (Most applications)

9. Confirm or change Set-up number on display. (Section 6.8)

10. Check analysis and inlet mode.

11. Print Set-up.

12. If using the Boat, connect Teflon tubing to inlet part of dehumidifier. (Fig. 4.15)

13. If using ASM, clean the rinse bottle and fill it with acidified DI water. (Few Drops of  $H_3PO_4$ )

14. Observe for stable baseline (Peak to Peak < 2mV) before starting analysis.

1. Check that system is not in the RUN mode.

2. Push **CARRIER** to turn off gas.

3. Leave furnace at operating temperature. (Normally 680°C)

4. Disconnect the Teflon tubing from the dehumidifier to boat at the boat inlet.

5. For total shut down turn OFF main power switch in the rear.

1. Select analysis mode (Table 6.1)

2. Select inlet mode (Table 6.2)

3. Confirm or change volume. (Fig. 6.1)

4. For CALIBRATION, press **CAL** to confirm or change concentration. (Section 6.7)

5. For manual injection, see Section 6.3 for injection technique.

6. For ASM, confirm or change other parameters. (Table 6.3)

7. Refer to Table 6.3 for ASM vial markers.

8. For RSM, see Section 6.5.

9. To complete CALIBRATION, see Section 6.7.

### Daily checks:

1. Printer paper
2. Gas supplies
3. IC chamber 1/2 full & acidified
4. Water in dehumidifier tube
5. Acid bottle 1/3 full
6. Gas flow 180-220cc/min
7. Temp. at set point
8. Dehumidifier temp 0-10°C

### Weekly checks:

1. Daily checks plus
2. Replace septum in POC sparger every 40 injections.
3. Inspect TC Inlet valve
4. Inspect combustion tube. Wipe inside area near top with wet Q-tips if necessary.
5. Inspect IC inlet valve
6. Clean IC reactor
7. Drain dehumidifier water & replace with acidified water. Flush several times if necessary.

### Monthly checks:

1. Daily & weekly and/or:
2. Inspect & replace UOH if necessary.
3. After ~ 160 hrs of operation, rinse catalyst, and combustion tube, replace silver wool (Section 7.1). Condition catalyst at 900°C for 1/2 hr with DI injections.
4. Inspect O-rings in TC inlet and bottom connector. Replace if necessary.

1. DO Check the bottom connector when checking the combustion tube.

2. DO Use a Soap Film Bubble meter to check output gas flow rates.

3. DO leave furnace at 680°C except for long term shut down.

4. DO Condition new catalyst. 100ul of water every 5 min. for 2 hours at 900°C.

5. DON'T use Pyrex wool in the combustion tube.

6. DO clean combustion tube weekly if used heavily. DI injections @ 900°C for 1/2 hrs. Use good water—should stabilize at 1 to 3ppm or better.

7. DO check valve seal & O-rings monthly when inspecting TC & IC ports.

8. DO re-align TC & IC ports with ASM probe after inspections.

9. DO study flow diagram Figs 8-1 & 8-2.

10. DO acidify ASM rinse bottle

11. DON'T use ASM stirring time > 30 sec.

12. DO inject acidified water daily into TC port if non-acidified samples are analyzed. (3, 100ul Inj. of pH1, HCl or  $HNO_3$ )

13. DO rinse (section 7.1) and condition catalyst (section 5.3) when catalyst is contaminated.

14. DON'T raise drain line higher than 1 1/2" above lab bench.



## SECTION 10

# STANDARDS PREPARATION AND SAMPLE HANDLING

---

### 10.1 STANDARDS PREPARATION

#### REAGENT WATER

Use:

Standards preparation, system blanks, sample dilution, cleaning, etc.

Requirements:

Deionized or distilled.

ASTM Type II reagent water or equivalent.

TOC level: Less than 0.2 mgC/L.

#### ACID SOLUTION

Use:

Automatic acid feed for IC chamber, sparge stations, autosampler.

Requirements:

Reagent water.

Phosphoric ( $\text{H}_3\text{PO}_4$ ), sulfuric ( $\text{H}_2\text{SO}_4$ ), or nitric ( $\text{HNO}_3$ ) acid, concentrated, reagent grade.

Do not use hydrochloric acid (HCl).

Preparation:

Final volume: 100 ml.

20% Phosphoric Acid Solution:

Add 20 ml acid to 80 ml reagent water. Transfer to the acid bottle (4 oz borosilicate with open top screw cap).

If phosphoric acid is not available, 10% sulfuric acid or 5% nitric acid can be substituted.

Replace monthly.



**TC and IC  
STOCK SOLUTIONS**

**Use:**

Dilute to appropriate concentration for calibration or system check-out.

**Requirements:**

Reagent water.

Reagent-grade concentrated acid ( $\text{H}_3\text{PO}_4$  or  $\text{H}_2\text{SO}_4$ ) for TC stock only.

Standard compounds are reagent-grade, and must be dried to a constant weight. (See the table in the next page.)

**Preparation:**

Final volume: 100 mL.

Standard compound choice:

For system performance check and troubleshooting purposes, use a compound listed below. For routine analyses, use one of these, or any compound which might be more appropriate for your application.

Weigh the specified amount of the compound into a 100 ml volumetric flask. Add about 75 ml reagent water to dissolve the compound. Add about 0.1 ml acid to TC solutions to adjust pH below 3. Then fill to the mark.

Store stock solutions in amber borosilicate bottles with Teflon-lined closures at 4°C.

Replace monthly.



---

**TC STOCK SOLUTIONS (Choose one):**

<u>Compound</u>	<u>Weight (g/100mL)</u>	<u>Concentration</u>	<u>Add Acid?</u>
KHP ( $C_8H_5KO_4$ )	2.126	10,000 mgC/L	Yes
Sucrose ( $C_{12}H_{22}O_{11}$ )	2.375	10,000 mgC/L	Yes

---

**IC STOCK SOLUTIONS (Choose one):**

<u>Compound</u>	<u>Weight (g/100mL)</u>	<u>Concentration</u>	<u>Add Acid?</u>
$Na_2CO_3$ (Anhydrous)	0.883	1,000 mgC/L	No
$NaHCO_3$	0.699	1,000 mgC/L	No

---

Use this formula to determine the weight required to make 100 ml stock solutions using other compounds:

$$g \text{ Compound} = \frac{mw \times \%C}{N \times 12.01}$$

**where:**

mw	=	molecular weight of compound
%C	=	concentration of standard in % carbon
N	=	number of carbon atoms per molecule
12.01	=	atomic weight of carbon

**For example**

For a 1% (10,000 mgC/L) solution of sucrose (mw = 342.29):

$$\frac{342.29 \times 1\%}{12 \times 12.01} = 2.375 \text{ g.}$$



**TC and IC  
WORKING STANDARDS**

**Use:**

Calibration or system check-out.

Choose the standard concentration to match the working range of your samples.

**Requirements:**

Reagent water.

Clean volumetric flasks and volumetric pipets.

**Preparation:**

Final volume: Depends on concentrations.

Use larger volumes as concentration decreases. Make 1 liter volume at 10 mgC/L. Do not make final volume smaller than 100 ml.

**TC solutions only:** Maintain at pH 3 or lower.

Store standard solutions in amber borosilicate bottles with Teflon-lined closures at 4°C. **Minimize exposure to atmosphere.**

Bottle volume: Between 100 - 200 mL, depending upon the concentration.

Replace weekly.

**System Performance Check:** (Initial Start-Up)

Make 100 ml of 1000 mgC/L TC standard and 100 ml of 100 mgC/L IC standard.



## POC STANDARD

### Use:

Calibrate POC sparger.

### Requirements:

Very clean 1 liter volumetric flask.

Reagent water.

Stir plate and Teflon coated stirbar.

Reagent grade compound.

### Preparation:

Final volume: 1000 ml.

Compound Choice:

**Benzene or chloroform is strongly recommended.** Other compounds can be used if reliable results can be demonstrated. Use only benzene or chloroform for system performance check and troubleshooting.

### WARNING!

#### **BENZENE**

**DANGER!** Extremely flammable.  
Suspected human carcinogen. Harmful if swallowed, inhaled or absorbed through the skin. May affect the blood system.

#### **CHLOROFORM**

**Warning!** Suspected human carcinogen. Harmful if inhaled or swallowed. Skin and eye irritant and may produce toxic vapors if burned.

Please consult material safety data sheets for more precautions regarding these compounds.

Fill the 1 liter flask to the mark with reagent water. Add the stir bar and gently agitate water on stirplate for 1 - 2 minutes to degas. Inject a microliter quantity of the compound. Use the table or formula in the following page to determine the proper quantity to inject. The syringe needle should be well immersed in the water. Cap the flask and gently agitate the solution until it comes to equilibrium (approximately 5 minutes).



**\* Less than 100 mgC/L**

- Use amber bottle.
- Wash in hot, soapy water.
- Rinse with clean water.
- Swirl with chromic/sulfuric acid cleaning solution.
- Rinse with reagent water.
- Use Teflon-lined cap.
- Store sample at 4°C.
- Analyze within two weeks.
- Treat standard bottles and sparge vials the same way.

**Sample Pretreatment:**

If a sample contains particulates larger than 0.5 mm or insoluble matter, homogenize with a blender or tissuemizer until the average particle size is less than 0.5 mm. Analyze these samples with the micropipettor or autosampler.

If the average particle size cannot be reduced to below 0.5 mm by homogenizing, dilute the sample with reagent water and blend again, or analyze the sample using the boat sampler.

**\* Below 100 mgC/L:**

Minimize the sample handling and the blend time in order to minimize contamination and loss of volatiles. Analyze a blank with the same pretreatment as a sample.



## 10.2 SAMPLE HANDLING

Good laboratory practice is important in obtaining reliable analysis for carbon content of samples. Since carbon is everywhere in nature, it is very easy to contaminate a sample. Follow these guidelines for sample handling during collection, pretreatment, and analysis.

### **Syringe Handling:**

Dedicate a syringe to a particular carbon range. When the syringe gets contaminated (indicated by sample or standard not completely wetting the inner barrel), draw chromic acid into the syringe a few times, then rinse well with reagent water.

### **Sample Bottles:**

It is preferable to store and collect samples in glass containers. Plastic bottles should only be used if it is established that the specific type of container to be used does not contribute contaminating organics.

The sample collection bottles should be cleaned well before collecting the sample. The amount of cleaning necessary is dependent on the expected concentration of carbon in the sample. As a rule of thumb, the following levels are suggested:

#### **\* Greater than 100 mgC/L**

- Wash bottle in hot, soapy water.
- Rinse with clean water.
- Plastic cap may be used, but try to use Teflon-lined cap.
- Analyze samples within 2 weeks.
- Treat standard bottles and sparge vials the same way.



COMPOUND	VOLUME TO INJECT	CONCENTRATION
Benzene (C <sub>6</sub> H <sub>6</sub> )	12 ul	9.92 mgC/L
Chloroform (CHCl <sub>3</sub> )	67 ul	9.72 mgC/L

To make other concentrations or standards, use this formula:

$$\text{Concentration of POC Standard } C = \frac{V \times D \times F}{L}$$

where:

- C = Concentration of standard (mgC/L)
- V = Microliters of POC solvent injected
- D = Density of POC solvent (mg/ul)
- F = Fraction of carbon per molecule by weight
- L = Volume in liters of water



## **Appendix B-15 – Lab Procedures for Chain of Custody**



**Table of Contents – Appendix B-15**

<b>Documents</b>
<b>TVA Sample Chain of Custody</b>



# TENNESSEE VALLEY AUTHORITY

NO.: SR-0001

TITLE: SAMPLE CHAIN OF CUSTODY

SIGNATURE	TITLE	DATE
Prepared By: <u>W. J. Rogers</u> <i>W. J. Rogers</i>	QA Officer	01-10-96
Concurred:		
Concurred:		
Concurred:		
Concurred:		
Concurred:		
Approved: <u>Joseph J. Woodland</u> <i>Joseph J. Woodland</i>	Manager, Environmental Applications	01-10-96

REVISION	01			
CONTROL DATE:	01-10-96			

COPY NO.: \_\_\_\_\_ HAS BEEN ISSUED TO HOLDER ON \_\_\_\_\_



## 1.0 PURPOSE

This procedure provides instructions for sample custody from collection to final disposition.

## 2.0 SCOPE

This procedure applies to all samples collected under a sampling plan which requires documentation of sample custody.

## 3.0 SUMMARY

Requirements for documentation of sample collection and sample custody are specified.

## 4.0 REFERENCES

4.1 U. S. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," SW-846, 3rd Edition, Most Recent Update (September 1994)

"Preparation Aids for the Development of Category II Quality Assurance Project Plans," EPA/600/8-91/004, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

"Preparation Aids for the Development of Category III Quality Assurance Project Plans," EPA/600/8-91/005, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268

## 5.0 RESPONSIBILITIES

5.1 The laboratory team leader shall ensure that this procedure is followed.

5.2 The sampler shall follow this procedure to ensure sample integrity in the field.

5.3 The person transporting the samples shall follow the procedure to ensure sample integrity in transit.

5.4 The person receiving the samples shall follow this procedure to ensure sample integrity upon receipt and immediately following.

5.5 Laboratory analysts shall follow this procedure during sample analysis.



## 6.0 REQUIREMENTS

### 6.1 Prerequisites

6.1.1 Sample containers shall be cleaned to specifications of the sampling plan, or in their absence, to good commercial practice.

6.1.2 Sample containers shall have preservative added before sampling as required by the sampling plan.

### 6.2 Limitations and Actions

6.2.1 If the sampling organization has its own sampling procedure, sample custody procedure, labels, or custody forms, they may be substituted for the contents of this procedure as permitted by the sampling plan.

6.2.2 The number of persons handling samples from the time of sampling to receipt by the laboratory should be held to a minimum.

6.2.3 Samples shall be labeled by attaching tie-on tags, adhesive labels, or by writing on sample containers with indelible markers. Samples shall be labeled with sufficient information that they may be traced to sample collection logs, field sheets, or custody records. Choice of adhesive labels or indelible ink should take into consideration that samples may come into contact with melted ice or condensed moisture during shipment or storage.

6.2.4 Individual samples shall be sealed or sample shipping containers shall be sealed with a tamper-proof seal when they will be relinquished by TVA to a common carrier or if the sampling plan requires it. If the samples will remain in the custody of TVA employees from the time of sampling through transport to the laboratory or under lock and key (as in a locked vehicle or storage area) during this time, use of seals is not required.) However, even if seals are not required, their use is strongly urged on shipping containers if the sample is to change hands several times in transport.

### 6.3 Requirements

#### 6.3.1 Apparatus/Equipment

This procedure specifies no additional apparatus or equipment in addition to any sampling plan.

#### 6.3.2 Materials

6.3.2.1 Sample containers specified in the sampling plan shall be utilized.



6.3.2.2 Labels - Samples labels shall have an adhesive which does not readily release when containers become damp.

6.3.2.3 Custody Forms - Sample chain of custody forms shall be used to record custody of samples after sampling from relinquishment by the sampling organization through transport to receipt by the laboratory. The following information shall be supplied on the custody form:

- a. Project identification
- b. Sample collection date
- c. Sample identification
- d. Collection time
- e. Number of containers per sample identification code
- f. Requested analysis
- g. Sampling location
- h. Comments
- i. Signature of sample collector.

In addition the form shall contain an area so that each relinquishment and receipt of samples may be documented.

Sample custody forms are attached as appendices 10.1 and 10.2. Other forms specific to a given project may be developed as long as they contain the minimum information specified above.

Note: If sample collection time and location are already recorded on a field sheet or sampling log, that information need not be repeated on this form provided a copy of the sampling information is transmitted to the laboratory with the custody sheet.

6.3.2.4 Seals - Custody seals are narrow strips of adhesive paper used to demonstrate that a sample or shipping container have not been tampered with. They must tear before peeling from the surface of the container. Commercially-available seals designed for this purpose are strongly recommended. Unprinted adhesive labels from office supply sources may be substituted provided they do not peel from surfaces in such a manner that tampering is not evident.

6.3.2.5 Field Logbooks or Field Sheets - Sampling activities may be documented in field logbooks or field sheets designed for that purpose. When these are used, they shall contain:

- a. Project identification
- b. Sample collection date
- c. Sample identification
- d. Collection time
- e. Number of containers per sample identification code



- f. Reference to the sampling procedure
- g. Sampling location
- h. Comments
- i. Signature of sample collector.

## 7.0 PROCEDURE

### 7.1 Calibration None

### 7.2 Procedure Instructions

#### 7.2.1 Field Operations

7.2.1.1 Prior to sampling, label sample containers with an adhesive label or with indelible marker. (Note: If the sampling conditions require it, labels may be affixed after sampling and cleaning the outside of the container.)

7.2.1.2 Document sample information in a field log, field sheet, or the custody sheet if the first two are not provided.

7.2.1.3 Seal the sample container with an adhesive seal if the sampling plan requires it.

7.2.1.4 Complete a "Sample Chain of Custody" form.

7.2.1.4.1 If field logs or field sheets contain collection time and location, these items may be omitted from the form. In that case, draw a diagonal line in that column and attach a copy of the field logs or sheet so that the laboratory may have pertinent sampling information.

7.2.1.4.2 If a numbered seal is to be used on the shipping container, note that number in the comments section of the custody form.

7.2.1.4.3 If the shipping container is to be sealed, sign and date the "relinquished" are of the form.

7.2.1.5 Place the original copy of the paperwork in a plastic bag inside the shipping container. Retain one copy for field files. Transmit a third copy by separate courier, mail or fax to the laboratory.

7.2.1.6 Place samples in a shipping container. As required by the sampling plan, place ice (or commercial substitute) and a temperature test bottle in the container as well. Seal the shipping container if the sampling plan requires it. See also 6.2.4.

7.2.1.7 Deliver the container to be transported to the laboratory.



## 7.2.2 Laboratory Receipt

7.2.2.1 Inspect the seals. Open the shipping container. Inspect the sample custody form to ensure that it is correctly completed. Sign as receiver. Compare the shipping container contents to the information on the form.

7.2.2.2 If the "relinquished" blank is not completed and the person delivering the samples is present, have that person sign the "relinquished by." Otherwise write "Not completed", date and initial. If a person signs "relinquished by," provide that person a copy of the paperwork.

7.2.2.2 As required by the sampling plan, measure the temperature of any samples or temperature blanks and record that information on the custody sheet.

7.2.2.3 Communicate any errors, broken seals, missing seals, broken samples, differing identification numbers, extra samples, missing samples or misidentification to field personnel. Document all discussions by memorandum or database sample comment file. Document all problems and their resolution by memorandum or database sample comment file. If seals show signs of tampering, bring this to the attention of the group leader or team leader.

7.2.2.4 If samples are not immediately logged into the database, store them in a locked, refrigerated storage area as required by the sampling plan or project plan.

7.2.2.5 Log the samples in the laboratory database. Note laboratory sample identification in the margin of the custody form. Log field sample identification in the laboratory database. Log collection dates and times in appropriate fields of the database. Apply adhesive labels to sample containers when their size allows it. Alternately, write laboratory sample identification on samples with indelible markers.

Note: Samples which have been refrigerated often have condensed moisture which does not allow indelible ink or labels to be applied to containers without wiping them clean of moisture.

7.2.2.6 Following logging, store the samples store them in a locked, refrigerated storage area as required by the sampling plan or project plan.

## 7.2.3 Laboratory Custody

7.2.3.1 Samples in locked storage areas, being processed, or in autosampler trays are considered to be in the custody of the laboratory. When sampling plans require it, laboratory work areas shall be locked when unattended.

## 7.2.4 Sample Disposal



7.2.4.1 When customers request it, samples shall be returned to them following analysis.

7.2.4.2 Otherwise, dispose of samples after the time period specified in the sampling plan or project plan. If these do not specify a date, samples should be kept no longer than three months after all analyses are complete.

## 8.0 SAFETY

8.1 In handling samples, be aware of spills on outside of containers. Clean the exterior of containers as needed.

8.2 Wear a lab coat, gloves, and safety glasses when unpacking or packing samples.

8.3 Store flammable samples in an explosion-proof refrigerator.

## 9.0 NOTES

None

## 10.0 ATTACHMENTS AND APPENDICES

10.1 Sample custody form- RCRA

10.2 Sample custody form - General

END OF PROCEDURE



## Environmental Site Remediation • TVA Environmental Research Center • Muscle Shoals, Alabama 35660

TVA 29203 B (RG-CTR 4-94)



**Sample Chain of Custody  
Tennessee Valley Authority  
Environmental Applications CTR-1K Muscle Shoals, AL**

Project \_\_\_\_\_

Date of Collection \_\_\_\_\_

	Sample ID	Collection Time	Number of Containers	Analyses Requested	Location	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
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50						

Collector \_\_\_\_\_

Signature \_\_\_\_\_

Date and Time \_\_\_\_\_

Relinquishing \_\_\_\_\_

Receiving \_\_\_\_\_



## Appendix C – Toxicity Studies



**Table of Contents – Appendix C**

<b>Documents</b>
<b>ERC constructed Wetlands TNT/TDX Degradation: Toxicity Testing of Constructed Wetlands Aquarium Gravel - Oct. 1995</b>
<b>TNT/RDX Degradation Toxicity Study (Daphnids) - Nov 2, 1995</b>
<b>Assessing Sediment Quality in Jacob's Creek at the Paradise Fossil Plant – Dec 21, 1995</b>



December 7, 1995

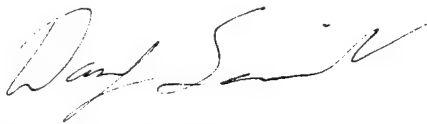
original to [unclear]  
Mater  
copy to [unclear]

H. Steven Coonrod, WET 1A-M

ERC CONSTRUCTED WETLANDS TNT/RDX DEGRADATION: TOXICITY  
TESTING OF CONSTRUCTED WETLANDS AQUARIUM GRAVEL - OCTOBER  
1995

Attached is a copy of the subject report for the aquarium gravel toxicity test conducted using amphipods, *Hyaella azteca*. Toxicity was not present in any gravel sample collected October 23, 1995 based on acute testing of amphipods.

Please call me at (205) 729-4805 if you have comments or questions following your review.



Damien J. Simbeck  
Biologist  
Toxicity Testing Laboratory  
TTL 1A-BFN

Attachments

cc (Attachments):

Gilberto Quintero, CC 1A-C  
Files, WM, CST 16C-C

Prepared by Damien J. Simbeck



## STANDARD REPORT FORM

### TOXICITY BIOMONITORING USING *HYALELLA AZTECA* (AMPHIPOD)

Test Title: ERC Wetlands TNT/RDX Degradation

Principal Investigator: Damien J. Simbeck

Starting Date: September 21, 1995

Ending Date: November 3, 1995

#### 1.0 EXECUTIVE SUMMARY

A study to determine the ability of gravel substrate wetlands to break down TNT/RDX compounds was conducted at TVA Environmental Research Center's Wetlands Laboratory. Toxicity studies were conducted utilizing the gravel substrates to determine if any toxic by-products of the TNT/RDX degradation process would adhere to the substrate, causing future problems in constructed wetlands. The amphipod, *Hyaella azteca*, was chosen as the toxicity test species, since it is an EPA approved test species and will readily utilize the gravel substrate for cover. They may also feed on the organic material attached to the gravel, increasing exposure to any degradation by-products.

Prior to testing the constructed wetlands substrate, a test was conducted using gravel from the same source as was used in the wetlands experiment. This test was conducted to determine if the test organisms could adequately survive and grow during a ten day period in the gravel substrate. Results of this test showed survival and growth similar to that which has been seen in past tests using *Hyaella azteca* in formulated sediment controls.

No adverse affects were observed for *Hyaella azteca* during a 10-day exposure to gravels from several simulated constructed wetlands. Survival and growth of the test organisms in all treatments exceeded that in the control gravel. Based on this information, it is likely that toxic by-products from the TNT/RDX degradation process would not adversely affect sediment dwelling, aquatic organisms.

#### 2.0 SAMPLE COLLECTION/TREATMENT

##### 2.1 Control Gravel Screening Test

2.1.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test substrates used for biomonitoring were unused gravels from TVA's Wetlands laboratory at the Environmental Research Center (ERC), Muscle Shoals, Alabama.



- 2.1.2 Control/Overlying and Dilution Water: Synthetic water with dechlorinated tap water (1:1 v:v) was used for dilution and control/overlying water.
- 2.1.3 Sample Dates and Times: August 26, 1995, 1500 CDT
- 2.1.4 Sampling Method: Samples were grab samples gathered from the gravel storage pile.
- 2.1.5 Sample Storage/Handling: Samples were placed in 1-gal plastic jars and stored dry at room temperature at TVA's Toxicity Testing Laboratory (TTL), Athens, Alabama.
- 2.1.6 Sample Transport: Samples were picked up at ERC and delivered to TTL by TTL personnel.
- 2.1.7 Sample Pretreatment: Samples were placed into test beakers on September 20, 1995 (Day -1), covered with 175 ml overlying water and placed into test system.
- 2.1.8 Test Treatments: Gravel only
- 2.2 Constructed Wetlands Aquarium Gravel Test
  - 2.2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test substrates used for biomonitoring were gravels from each of six constructed wetlands used to treat water spiked with TNT/RDX at TVA's Wetlands laboratory at the Environmental Research Center (ERC), Muscle Shoals, Alabama.
  - 2.2.2 Control/Overlying and Dilution Water: Synthetic water with dechlorinated tap water (1:1 v:v) was used for dilution and control/overlying water.
  - 2.2.3 Control Substrate: Clean, unused gravel from ERC's Wetlands laboratory.
  - 2.2.4 Sample Dates and Times: October 23, 1995, 1100 CDT; Control gravel was collected August 26, 1995, 1500 CDT.
  - 2.2.5 Sampling Method: Test samples were grab samples gathered when the wetlands were disassembled.
  - 2.2.6 Sample Storage/Handling: Samples were placed on ice in doubled zip-lock bags in coolers for shipment to TVA's Toxicity Testing Laboratory (TTL), Athens, Alabama. Control gravel was collected and stored in 1-gal plastic jars.



- 2.2.7 Sample Transport: Test samples were delivered to TTL by Wetlands personnel immediately after collection.
- 2.2.8 Sample Pretreatment: Samples were placed into test beakers when received (Day -1), covered with 175 mL overlying water and placed into test system.
- 2.2.9 Test Treatments: Gravel from the following constructed wetlands:

Aquarium Number	Plant Species	Plant Density	Nutrient Level
4	Canary Grass	50	700
10	Canary Grass	50	700
11	Canary Grass	50	350
13	Parrot Feather	50	700
15	Canary Grass	50	350
20	Parrot Feather	50	700

### 3.0 *Hyalella azteca*, AMPHIPOD /CULTURE CONDITIONS

- 3.1 Source: In-house culture, TVA, Toxicity Testing Laboratory
- 3.2 Culture Water: Culture water consists of moderately hard synthetic water mixed with dechlorinated tap water (1:1 v/v). Reagents for synthetic water were added to Milli-Q UF product water. Culture water was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 110 mg/L as CaCO<sub>3</sub>.
- 3.3 Temperature of Culture: 23°C ± 2°C
- 3.4 Test Organism Isolation: Animals of correct test age (7-14 days old) were obtained by sieving. Amphipods which pass through a US Standard #35 (500 µm) sieve, but are stopped by a #45 (355 µm) sieve are the mean size of 6-d old amphipods [1]. Amphipods for the gravel test were isolated on September 20, 1995. Amphipods for the aquarium test were isolated daily October 18-23, 1995.
- 3.5 General Maintenance: Test organisms were held in a 20-L aquaria with approximately 10-L culture water. Approximately 2 L culture water was exchanged daily. Organisms were fed 5-mL YCT daily after water renewal. The holding temperature of test organisms used for the Aquarium Test was 23.0°C (22.0°C-23.7°C).
- 3.6 Age at test initiation: Gravel Test: 7 days  
Aquarium Test: 7-12 days



4.0 Amphipod, *Hyalella azteca*, Survival test, EPA Test Method 100.1 [1]

- 4.1 Modifications/Deviations to 100.1:
- 4.1.1 Water feed rates varied more than 10% among replicates using the syringe delivery system during system calibration for both tests. Test beakers were therefore rotated daily within the system so that all replicates would receive water from each syringe during the test.
- 4.1.2 Water was renewed only once on October 28, 1995 during the aquarium test.
- 4.1.3 During the gravel test, the temperature on Day 2 was 19.2°C which was below the EPA recommended range of 23°C  $\pm$  3°C. The overall temperature range for the test was 22.7°C, which was within the EPA recommended average. This deviation was judged to have no impact on test results.
- 4.1.4 During the aquarium test, total residual chlorine (TRC) was measured at 0.4 mg/L in the overlying water used for the morning renewal on Day 8, due to the failure to check medium quality before use. Aeration caused by pouring, splashing, etc. during sample warm-up and pour-up probably removed this TRC. The TRC before the afternoon renewal on Day 8 was <0.1 mg/L. It is believed this caused no adverse affects to the test organisms since test acceptability criteria for survival and growth were met in controls.
- 4.2 Date/Time Test Initiated: Gravel Test: September 21, 1995;  
0905-0915 CDT  
Aquarium Test: October 24, 1995,  
1030-1045 CDT
- 4.3 Date/Time Test Terminated: Gravel Test: October 1, 1995;  
0810-0900 CDT  
Aquarium Test: November 3, 1995;  
0720-1350 CST
- 4.4 Age of Test Organism: Gravel Test: 7 days  
Aquarium Test: 7-12 days
- 4.5 Test Chamber: 400 mL beaker with 350  $\mu$ m Nitex® mesh covering a notch at the top.
- 4.6 Volume in Chamber: 100 mL gravel, 175 mL water
- 4.7 Number of Organisms per Replicate: 10
- 4.8 Number of Replicates per Treatment: 8



- 4.9 Test Control Substrate: Clean gravel
- 4.10 Dilution Substrate: NA
- 4.11 Overlying Water: Moderately hard synthetic water plus dechlorinated tap water, mixed 1:1 (v:v).
- 4.12 Dilution water: NA
- 4.13 Test Temperature:  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (Instantaneous temperature  $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ )
- 4.14 Photoperiod: 16 L:8D
- 4.15 Renewal Period: Overlying water renewed twice daily by slow flow delivery system.
- 4.16 Renewal Method: Water was fed from a head tank with eight 10-mL syringes to supply slow flow (about 200 mL/10 minutes) into each test chamber.
- 4.17 Feeding Regime During Test: 1.5 mL/replicate YCT once daily. YCT prepared according to EPA/600/4-89/001. [2]
- 4.18 Test Aeration: During the aquarium test, dissolved oxygen levels on Day 0 were below the TTL minimum of 4 mg/L in Aquarium 20. All treatments were aerated with a slow, steady bubble in each replicate throughout the test. It was determined that this caused no adverse affects to the test organisms, since test acceptability requirements for survival and growth were met in the controls.
- 4.19 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions were temperature (adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness and total residual chlorine.

"Final" measurements of temperature, was taken in one replicate per treatment before renewal. "Final" measurements of DO, pH and conductivity were taken daily and alkalinity and hardness were taken on Days 1, 5 and 10 in a composite of 30-50 mL samples removed daily from all replicates per treatment before renewal. On Days 1, 5 and 10, test solutions (100 mL) were preserved with 1:4  $\text{H}_2\text{SO}_4$  and refrigerated until sent to TVA's Environmental Chemistry Laboratory in Chattanooga Tennessee, for ammonia analysis using the automated alkaline phenate methodology. No final alkalinity, hardness or ammonia measurements were made during the gravel screening test.



- 4.20 Test Endpoint Determination:
- 4.20.1 Survival: Test animals were counted as dead if they could not be found at test termination
- 4.20.2 Growth: Growth was determined by dry weight measurements after test termination.
- 4.21 Statistics: Revised statistical procedures contained in the fourth edition of EPA's acute toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [2] No statistical analyses could be conducted from the gravel test due to lack of additional treatments. Statistical analyses were not necessary for the aquarium test since survival and growth for all treatments were greater than the control.

## 5.0 QUALITY ASSURANCE

- 5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-89/001. [3][2] Any known deviations were noted during the study and are reported herein.
- 5.2 Physical and Chemical Methods
- 5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.
- 5.2.2 Instruments: All identification, service and calibration information pertaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.
- 5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [4]



- 5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [4]
- 5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [4]
- 5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [4]
- 5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H<sub>2</sub>SO<sub>4</sub> to an endpoint of 4.5 according to TVA procedure ES-42.1. [4]
- 5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colormetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.
- 5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9. [4]
- 5.3 Reference Toxicant Tests
- 5.3.1 Test Type: Amphipod -- 96-hr acute, water only (LC<sub>50</sub>)
- 5.3.2 Standard Toxicant Used: Potassium Chloride (KCl)
- 5.3.3 Dilution Water Used: Moderately hard synthetic water with 50% dechlorinated tap water
- 5.3.4 Statistics: Probit, Spearman-Kärber, etc.

## 6.0 RESULTS

- 6.1 Summary of Bioassay Results:
- 6.1.1 Gravel Test
- 6.1.2 Summary of Results: Ten day exposure of amphipods to the gravel from TVA's Wetlands laboratory showed acceptable survival and growth based on past tests conducted at TTL.



6.1.3 Ten-day Percent Survival Summary for *Hyaella azteca*, Wetlands TNT/RDX Gravel Test, September 21-October 1, 1995.

Treatment	Amphipod Survival Data (% Survival)								Mean
	Replicate								
	1	2	3	4	5	6	7	8	
Control	90	100	100	80	90	100	90	80	91.6

6.3.3 Ten-day Growth Summary, *Hyaella azteca*, Wetlands TNT/RDX Gravel Test, September 21-October 1, 1995.

Treatment	Amphipod Dry Weight (mg)								Mean
	Replicate								
	1	2	3	4	5	6	7	8	
Control	0.094	0.135	0.093	0.169	0.174	0.159	0.079	0.071	0.122

6.1.1 Aquarium Test

6.1.2 Summary of Results: Ten day exposure of amphipods to the gravel from constructed wetlands demonstrated no toxicity (survival or growth) from any aquarium tested.

6.1.3 Ten-day Percent Survival Summary for *Hyaella azteca*, Wetlands TNT/RDX Aquarium Test, October 24-November 3, 1995.

Amphipod Survival Data (% Survival)									
Treatment	Replicate								Mean
	1	2	3	4	5	6	7	8	
Control	90	60	90	70	90	90	90	90	83.8
Aquarium 4	100	90	90	100	80	80	90	90	90.0
Aquarium 10	100	70	100	100	60	100	100	100	91.3
Aquarium 11	80	100	100	100	90	90	100	90	93.8
Aquarium 13	90	100	80	90	90	80	80	100	88.8
Aquarium 15	100	90	100	80	80	90	100	100	92.6
Aquarium 20	100	90	100	80	100	90	80	90	91.4



6.3.3 Ten-day Growth Summary, *Hyalella azteca*, Wetlands TNT/RDX Aquarium Test, October 24-November 3, 1995.

	Amphipod Dry Weight (mg)								
	Replicate								
Treatment	1	2	3	4	5	6	7	8	Mean
Control	0.034	0.093	0.103	0.079	0.061	0.057	0.058	0.059	0.068
Aquarium 4	0.237	0.199	0.218	0.129	0.146	0.189	0.141	0.243	0.203
Aquarium 10	0.080	0.074	0.153	0.113	0.082	0.138	0.096	0.117	0.107
Aquarium 11	0.165	0.129	0.152	0.105	0.117	0.160	0.189	0.089	0.151
Aquarium 13	0.108	0.102	0.123	0.136	0.133	0.168	0.150	0.106	0.128
Aquarium 15	0.102	0.189	0.134	0.105	0.170	0.169	0.163	0.180	0.153
Aquarium 20	0.086	0.059	0.121	0.162	0.154	0.113	0.139	0.158	0.124

6.5 Physical/Chemical Parameters

6.5.1 Overall Test Temperature: Gravel Test: 22.7°C (19.2°-24.8°)  
 Aquarium Test: 22.3°C (20.3°-23.7°)

6.6 Results: Water chemistry summary for ERC-TNT/RDX Gravel and Aquarium Tests, September 21-October 1, 1995 and October 24-November 3, 1995.

See: Appendix A Water Chemistry Mean Values and Ranges for ERC-TNT/RDX Gravel Test, September 21-October 1, 1995.

See: Appendix B Water Chemistry Mean Values and Ranges for ERC-TNT/RDX Aquarium Test, October 24-November 3, 1995.

6.7 Reference Toxicant Tests

6.7.1 Summary of Results:

The most recent amphipod reference toxicants tests conducted prior to ERC-TNT/RDX Gravel and Aquarium Tests showed acute results consistent with prior tests at TTL as well as those published in EPA 600/R-94/024. [1]



6.7.2 Date/Time of Most Recent Test: Test 1 September 20-24, 1995/0800 CDT  
Test 2 October 17-21, 1995/1330 CDT

6.7.3 LC<sub>50</sub>: Test 1: 288 (95% C.L. = 217-386) mg/L KCl  
Test 2: 270 (95% C.L. = 205-361) mg/L KCl

6.7.4 TTL Test Summary:

Number of standard tests completed by laboratory: 7

LC<sub>50</sub> Range: 270-354 mg/L KCl

6.7.5 EPA Test Summary:

Number of laboratories conducting tests: 9

LC<sub>50</sub> Mean: 305.0 mg/L KCl

Laboratory Ranges: 232.0-372.0 mg/L KCl

## 7.0 CONCLUSIONS

7.1 No adverse affects were observed for *Hyalella azteca* during a 10-day exposure to gravels from several simulated constructed wetlands. Survival and growth of the test organisms in all treatments exceeded that in the control gravel. Based on this information, it is likely that toxic by-products from the TNT/RDX degradation process would not adversely affect sediment dwelling, aquatic organisms.



## 8.0 REFERENCES

1. U. S. EPA, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, EPA 600/R-94/024 (June 1994)
2. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Mendick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
3. Toxicity Testing Laboratory Quality Assurance Program and Standard Operating Procedures Manual, Division of Water Resources, Tennessee Valley Authority (October 1992).
4. Field Operations Natural Resources Engineering Procedure Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.



# Appendix A

## Water Chemistry Mean Values and Ranges for ERC TNT/RDX Degradation Gravel Test, September 21-October 1, 1995

Source	Temperature Final	Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness		Chlorine	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*		*		(mg/L)	
Overlying Water	-	8.0 (8.0-8.0)	-	8.2 (8.2-8.2)	-	290 (290-290)	-	72 (72-72)	-	96.0 (96.0-96.0)	-	<0.1 (<0.1-<0.1)	-
Gravel <i>H. azteca</i>	22.7 (19.2-24.8)	-	7.4 (6.7-8.5)	-	7.7 (7.0-7.8)	-	311 (301-321)	-	-	-	-	-	-

\* mg/L as CaCO<sub>3</sub>



# Appendix B

## Water Chemistry Mean Values and Ranges for ERC TNT/RDX Degradation Aquarium Test, October 24-November 3, 1995

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness		Unionized NH <sub>4</sub>		Chlorine	
	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial
Overlying Water	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	*	*	*	(mg/L)	(mg/L)	(mg/L)	(mg/L)
	-	8.7 (8.6-8.9)	-	8.2 (8.1-8.2)	-	312 (297-317)	-	EC (79-80)	-	105.2 (104.0-110.0)	-	-	-	-	-	<0.1 (<0.1-0.4**)
Gravel	22.9 (21.0-23.7)	-	8.1 (7.9-8.6)	-	8.1 (7.7-8.2)	-	324 (311-335)	-	74 (70-77)	-	108.7 (104.0-112.0)	-	0.040 (0.031-0.054)	-	-	-
<i>H. azteca</i>																
Aquarium 4	22.3 (20.4-23.2)	-	7.6 (4.3-8.2)	-	8.0 (7.4-8.1)	-	315 (301-328)	-	74 (66-84)	-	108.7 (108.0-110.0)	-	<0.004 (<0.001-0.008)	-	-	-
<i>H. azteca</i>																
Aquarium 10	22.1 (20.3-22.8)	-	7.7 (4.3-8.7)	-	7.9 (7.3-8.1)	-	310 (288-324)	-	70 (64-76)	-	106.0 (104.0-108.0)	-	<0.003 (<0.001-0.004)	-	-	-
<i>H. azteca</i>																
Aquarium 11	22.1 (20.4-22.8)	-	7.6 (4.7-8.2)	-	8.0 (7.4-8.1)	-	317 (306-329)	-	79 (75-88)	-	110.0 (108.0-112.0)	-	<0.002 (<0.001-0.002)	-	-	-
<i>H. azteca</i>																
Aquarium 13	22.0 (20.4-22.7)	-	7.8 (4.0-8.7)	-	8.0 (7.3-8.1)	-	312 (283-327)	-	73 (70-78)	-	108.0 (108.0-108.0)	-	<0.003 (<0.001-0.005)	-	-	-
<i>H. azteca</i>																
Aquarium 15	22.1 (20.3-22.8)	-	7.8 (4.7-8.3)	-	7.9 (7.4-8.1)	-	311 (293-325)	-	74 (70-79)	-	108.0 (106.0-110.0)	-	<0.002 (<0.001-0.003)	-	-	-
<i>H. azteca</i>																
Aquarium 20	22.1 (20.4-23.1)	-	7.5 (3.8-8.3)	-	7.9 (7.3-8.1)	-	318 (298-331)	-	76 (73-80)	-	110.0 (108.0-114.0)	-	<0.005 (<0.001-0.010)	-	-	-
<i>H. azteca</i>																

\* mg/L as CaCO<sub>3</sub> \*\*see Section 4.2.4.



November 2, 1995

Steve Coonrod, WET 1A-M

TNT/RDX DEGRADATION TOXICITY STUDY (DAPHNIDS)

Attached are copies of the subject reports for the toxicity studies of the constructed wetlands degradation on TNT/RDX. Toxicity was demonstrated in all water-only wetlands and in three of the six gravel wetlands. All toxicity in the gravel wetlands was based on results of hypothesis testing. Current EPA recommended statistical analyses use  $IC_{25}$  values (25% reduction in survival and reproduction) to denote toxicity. Serial dilutions must be tested for  $IC_{25}$  analyses, however, since no survival effects were demonstrated in the wetlands study, and reproduction was not reduced greater than 25% in the gravel wetlands, these treatments probably did not demonstrate biologically significant toxicity.

Please call me at (205) 729-4805 if you have comments or questions following your review. All data from these studies will be kept as confidential files at TTL, and will be released only with your written permission.



Damien J. Simbeck  
Biologist  
Toxicity Testing Laboratory  
TTL 1A-BFN

Attachments

cc (Attachments):

G. Quintero, CC 1A-C (Confidential Files)

Prepared by Damien J. Simbeck



TENNESSEE VALLEY AUTHORITY  
TOXICITY TESTING LABORATORY (TTL)  
STANDARD REPORT FORM

TOXICITY BIOMONITORING USING  
*CERIODAPHNIA DUBIA* (DAPHNIDS)

Test Title: ERC-Wetlands TNT/RDX Degradation, Fourth Quarter, 1995

Principal Investigator: Damien J. Simbeck

Report Preparation: Damien J. Simbeck

Starting Date: September 29, 1995

Ending Date: October 6, 1995

1.0 EXECUTIVE SUMMARY

Toxicity testing of the outflow water from simulated constructed wetland aquaria was conducted to determine if chronic toxicity was present in water samples containing TNT/RDX after they had passed through different constructed wetlands. The organism specified for testing was the daphnid (*Ceriodaphnia dubia*).

Tests conducted using outflow water samples collected September 28-October 4, 1995, demonstrated toxicity (reduced reproduction) to daphnids in samples from aquaria 2, 6, 16 and 19 (water-only wetlands) and aquaria 10, 13, and 15 (gravel wetlands). No toxicity (survival or reproduction) was demonstrated in samples from aquaria 4, 11, or 20 (all gravel wetlands). Current EPA recommended statistical analyses use IC<sub>25</sub> values (25% reduction in survival and reproduction) to denote toxicity. Since survival in all treatments was 100%, and reproduction in the gravel wetlands water samples was reduced less than 25%, these samples probably did not demonstrate biologically significant toxicity.

2.0 SYSTEM OPERATION

- 2.1 Location/Description: The simulated constructed wetlands were located at TVA' Environmental Research Center (ERC) Wetland Research building in Muscle Shoals, Alabama. Simulated constructed wetlands consisted of 37.85 L glass aquaria.



## 2.2

## Aquaria Description:

Aquarium Number	Substrate	Plant Species	Plant Density	Nutrient Level
2	Water	Parrot Feather	50	700
4	Gravel	Canary Grass	50	700
6	Water	Parrot Feather	50	350
10	Gravel	Canary Grass	50	700
11	Gravel	Canary Grass	50	350
13	Gravel	Parrot Feather	50	700
15	Gravel	Canary Grass	50	350
16	Water	Parrot Feather	25	350
19	Water	Parrot Feather	25	700
20	Gravel	Parrot Feather	50	700

3.0 SAMPLE COLLECTION/TREATMENT

- 3.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The samples used for biomonitoring were the outflow water from each aquaria. Water samples consisted of 24-hr composite samples collected daily from each aquaria.
- 3.2 Control and/or Dilution Water: Modified Moderately Hard Synthetic Water was used for dilution and control (see Section 4.3).
- 3.3 Test Treatments: Outflow samples were tested at 100% only for each aquaria.
- 3.4 Sample Collection and Use: Test samples were collected daily, transported to TTL and used the following day. Samples were collected at 1500 CDT each day, September 28-October 4, 1995.
- 3.5 Sampling Method: All samples were 24-h composite samples collected continuously using Neoprene® tubing connected to each outflow pipe. Samples were collected in new rinsed Nalgene bottles placed on ice in a cooler. Flow rates from aquaria were approximately 1-L per day. Excess air was pressed from the bottles before lids were put in place.



- 3.6 Sample Storage/Handling: All samples were placed on ice in an ice chest after excess air was removed from the bottles. All samples remained on ice until used.
- 3.7 Sample Transport: Samples collected each day were taken to TVA's Public Safety office in Muscle Shoals by Wetlands personnel where they were picked up and delivered to TTL by TTL personnel.
- 3.8 Sample Pretreatment: Samples were filtered through a 64- $\mu$ m nylon mesh filter into 600-mL beakers. Sample temperature was raised to 25°C in a warm water bath and aerated as necessary to bring DO levels to near 100 percent saturation.

#### 4.0 TEST ORGANISMS/CULTURE CONDITIONS

- 4.1 Species: Ceriodaphnia dubia, daphnid
- 4.2 Source: In-house culture, TVA, Toxicity Testing Laboratory
- 4.3 Culture Water: Culture medium is moderately hard synthetic water containing trace elements, macronutrients, and vitamins (modified from Elenöt and Bias, 1990). [1] Water used for culture contains EDTA, while water used for test control and dilution does not.
- 4.4 Temperature of Culture: 25°C  $\pm$  1°C
- 4.5 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates from broods as described below on 2 or 3 consecutive days, 6-10 days prior to test initiation. Adults up to 14 days old may be used for neonate production. These animals are raised individually, and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass culturing may also be required at times to maintain sufficient brood stock for supporting the above. Mass cultures are maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adults, from broods containing 8 or more young.



- 4.6 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/013 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [2]

In addition to the yeast/alfalfa fish food recipe, the alga Selenastrum capricornutum concentrated to  $30 \times 10^6$  cells/mL is also fed as part of the regular diet. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal on 0.1 mL food recipe only on intermediate days.

## 5.0 TEST METHODS

- 5.1 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0 [2]
- 5.2 Modifications/Deviations to Method 1002.0:
- 5.2.1 Modified synthetic water (see Section 4.3)
- 5.2.2 Prepared Food recipe (see Section 4.6)
- 5.2.3 Feeding regime (see Section 5.11)
- 5.3 Date/Time Test Initiated: September 29, 1995/1415-1445 CDT
- 5.4 Date/Time Test Terminated: October 6, 1995/1415-1425 CDT
- 5.5 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)  
Volume per Chamber: 15 mL
- 5.6 Number of Organisms per Chamber: 1
- 5.7 Number of Replicates per Treatment: 10
- 5.8 Control/Dilution Water: Modified moderately hard synthetic water
- 5.9 Renewal period: 24-h
- 5.10 Test Temperature:  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- 5.11 Feeding Regime During Test: Each organism was fed 0.1 mL prepared food and 0.2 mL algae concentrate daily (added to renewal water before introduction of test organism).



- 5.12      Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Final measurements of temperature were taken daily in 10 randomly selected cups when the test tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 7.7.
- 5.13      Statistics: Fisher's Exact Test was used to determine significant survival effects, if applicable. EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [2] The reproduction data was analyzed in two parts, grouped according to substrate type (water or gravel). Both parts were compared to the same control. The decision process followed for testing sublethal (reproduction) effects is shown in sections 7.4.1 and 7.6.1, . Based on tests for normally distributed and homogenous variances, the statistical test used for reproduction data endpoint determination was Dunnett's Test (Hypothesis Test) for all aquaria.

## 6.0      QUALITY ASSURANCE

- 6.1      Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-89/001. [2] Any known deviations were noted the study and are reported herein.
- 6.2      Physical and Chemical Methods
- 6.2.1      Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.
- 6.2.2      Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.



- 6.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [3]
- 6.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [3]
- 6.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [3]
- 6.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [3]
- 6.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N  $\text{H}_2\text{SO}_4$  to an endpoint of 4.5 according to TVA procedure ES-42.1. [3]
- 6.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.
- 6.3 Reference Toxicant Tests
- 6.3.1 Test Type: Daphnids -- 7-day chronic (NOEC,  $\text{IC}_{25}$ )
- 6.3.2 Standard Toxicant Used: Sodium Chloride (NaCl crystalline)
- 6.3.3 Dilution Water Used: modified moderately hard synthetic water - daphnid chronic
- 6.3.4 Statistics: Chronic Test, NOEC - Dunnett's or Steel's Many-One Rank Tests,  $\text{IC}_{25}$  - EPA Bootstrap Procedure



## 7.0 RESULTS

### 7.1 Ceriodaphnia Survival and Reproduction Test

7.2 Summary of Results: Exposure of daphnids to outflow water samples collected September 28-October 4, 1995, resulted in toxicity (reduced reproduction) in samples from aquaria 2, 6, 10, 13, 15, 16, and 19. No toxicity (survival or reproduction) was demonstrated in samples from aquaria 4, 11, or 20. Since survival in all treatments was 100%, and reproduction in the gravel wetlands water samples was reduced less than 25%, these samples probably did not demonstrate biologically significant toxicity.

### 7.3 Results, Survival Data-Aquarium Test, Water Substrate:

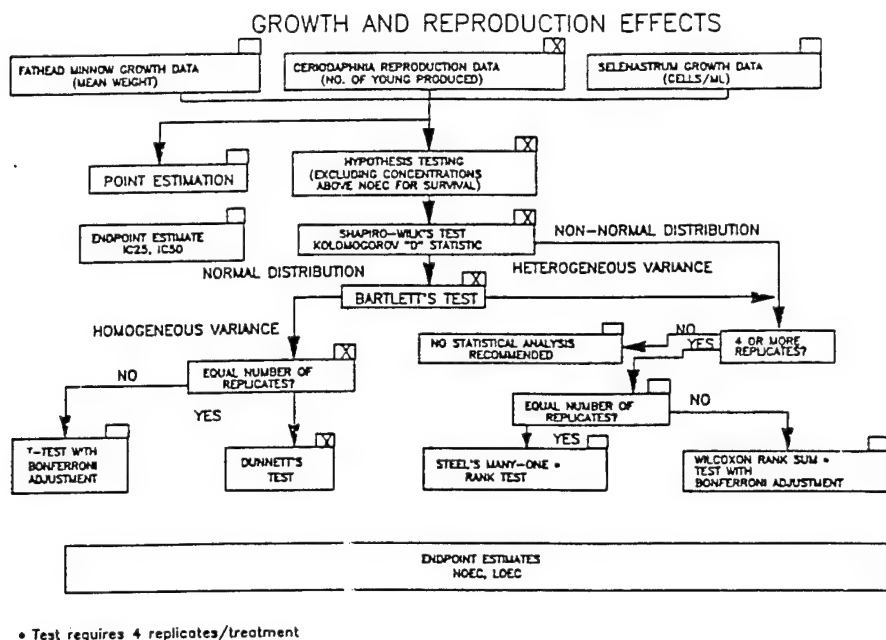
Daily Percent Survival Summary for *Ceriodaphnia* Survival Test, ERC-Wetlands TNT Degradation Test, Water Substrate, September 29-October 6, 1995.

Treatment	Total Daily % Survival						
	Day 1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
Aquarium 2	100	100	100	100	100	100	100
Aquarium 6	100	100	100	100	100	100	100
Aquarium 16	100	100	100	100	100	100	100
Aquarium 19	100	100	100	100	100	100	100



## 7.4 Results, Reproduction Data-Aquarium Test-Water Substrate:

### 7.4.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-day Exposure of Daphnids in ERC-Wetlands TNT Degradation Samples-Water Substrate (Aquaria 2, 6, 16, and 19)



### 7.4.2 Analysis of Reproduction (#young/female/7 days) Data Using Dunnett's Test for *Ceriodaphnia* Survival Tes., ERC-Wetlands TNT Degradation Test-Water Substrate, September 29-October 6, 1995.

No transformation applied before analysis. For this set of data, the minimum significant difference is 3.65.

This represents a 10.45% reduction in Reproduction (#young/female/7 days)  
 $T = 2.22$        $\text{Alpha} = 0.05$  (one-tailed test)

Reproduction (# young/female/7 days) Data												
Treatment	Replicate										Mean	S*
	1	2	3	4	5	6	7	8	9	10		
Control	40	35	35	34	35	37	36	32	36	29	34.9	
Aquarium 2	23	19	22	18	16	26	26	26	15	13	20.4	*
Aquarium 6	16	25	20	21	22	23	20	21	19	14	20.1	*
Aquarium 16	19	16	25	14	22	14	21	21	24	20	19.6	*
Aquarium 19	3	9	3	0	5	0	2	7	4	8	4.1	*

\*S - Statistically significant from control  
 EMS = 13.5



## 7.5

## Results, Survival Data-Aquarium Test-Gravel Substrate:

Daily Percent Survival Summary for *Ceriodaphnia* Survival Test, ERC-Wetlands TNT Degradation Test-Gravel Substrate, September 29-October 6, 1995.

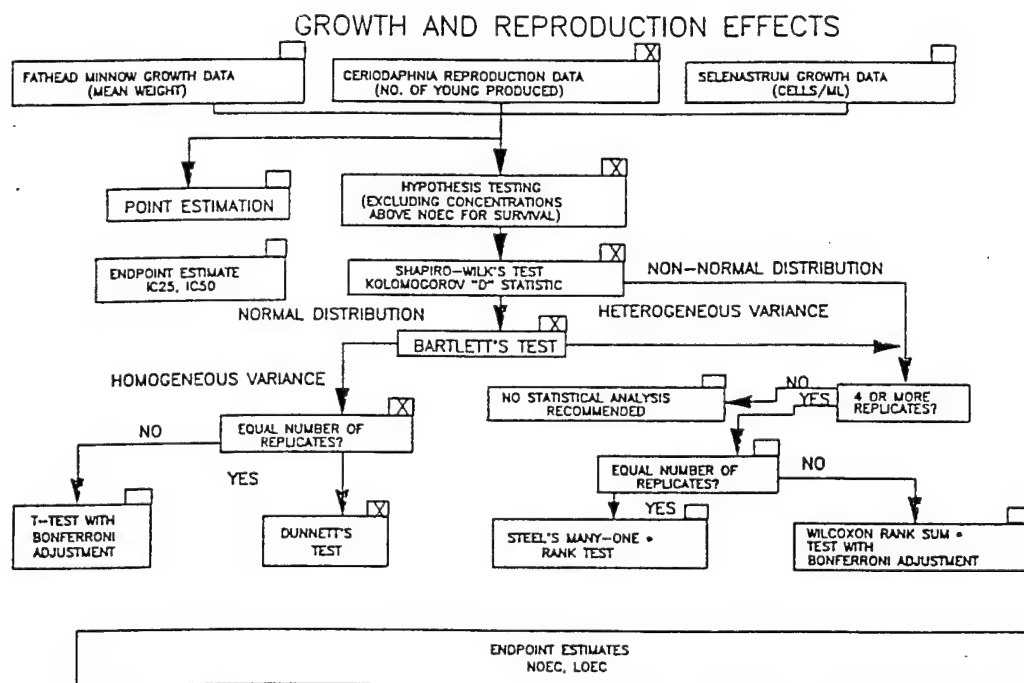
Treatment	Total Daily % Survival						
	Day 1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
Aquarium 6	100	100	100	100	100	100	100
Aquarium 10	100	100	100	100	100	100	100
Aquarium 11	100	100	100	100	100	100	100
Aquarium 13	100	100	100	100	100	100	100
Aquarium 15	100	100	100	100	100	100	100
Aquarium 20	100	100	100	100	100	100	100

## 7.6

## Results, Reproduction Data-Aquarium Test-Gravel Substrate:

## 7.6.1

Statistical Decision Process for Determining Toxicity Endpoints for 7-day Exposure of Daphnids in ERC-Wetlands TNT Degradation Samples-Gravel Substrate (Aquaria 4, 10, 11, 13, 15 and 20)



• Test requires 4 replicates/treatment



7.6.2 Analysis of Reproduction (#young/female/7 days) data Using Dunnett's Test for *Ceriodaphnia* Survival Test, ERC-Wetlands TNT Degradation Test-Gravel Substrate, September 29-October 6, 1995.

No transformation applied before analysis. For this set of data, the minimum significant difference is 3.13.

This represents a 8.98% reduction in Reproduction (#young/female/7 days)

T =2.35

Alpha = 0.05 (one-tailed test)

Reproduction (# young/female/7 days) Data												
Treatment	Replicate										Mean	S*
	1	2	3	4	5	6	7	8	9	10		
Control	40	35	35	34	35	37	36	32	36	29	34.9	
Aquarium 4	35	39	34	39	37	39	39	36	33	35	36.6	
Aquarium 10	23	29	29	34	26	29	31	27	26	26	28.0	*
Aquarium 11	31	30	29	35	37	34	37	30	29	30	32.2	
Aquarium 13	37	30	33	33	29	31	28	30	28	29	30.8	*
Aquarium 15	29	25	28	27	28	27	30	25	28	26	27.3	*
Aquarium 20	38	42	40	39	36	38	40	35	28	32	36.8	

\*S - Statistically significant from control

EMS = 8.9

7.8 Physical/Chemical Parameters

7.8.1 Overall Test Temperature: Mean = 25.0°C (24.1°C-25.7°C)

7.8.2 Results: Water chemistry summary for ERC-Wetlands TNT Degradation Study, September 29-October 6, 1995.

See: Appendix A-Water Chemistry Mean Values and Ranges for *Ceriodaphnia* Test, ERC-Wetlands TNT Degradation Study, September 29-October 6, 1995.

7.9 Reference Toxicant Tests

7.9.1 Summary of Results:

The most recent reference toxicant test conducted prior ERC-Wetlands TNT Degradation Study, September 29-October 6, 1995, showed chronic results consistent with TTL control chart ranges.



## 7.9.2 *Ceriodaphnia*

### 7.9.2.1 Chronic Tests

7.9.2.2 Date/Time of Most Recent Test: September 27-October 3, 1995/1010 CDT

7.9.2.3 NOEC: 900 mg NaCl/L  
LOEC: 1300 mg NaCl/L  
IC25: 1354 mg NaCl/L

### 7.9.2.4 Control Chart Information:

Number of standard tests completed by laboratory: 21

NOEC Range: 490-1300 mg NaCl/L  
LOEC Range: 900-1800 mg NaCl/L  
IC25 Range: 921-1574 mg NaCl/L (mean  $\pm$  two standard deviations)

## 8.0 CONCLUSION

Tests conducted using outflow water samples collected September 28-October 4, 1995, demonstrated toxicity (reduced reproduction) to daphnids in samples from aquaria 2, 6, 16 and 19 (water-only wetlands) and aquaria 10, 13, and 15 (gravel wetlands). No toxicity (survival or reproduction) was demonstrated in samples from aquaria 4, 11, or 20 (all gravel wetlands).

## 9.0 REFERENCES

1. Elendt, B. P., and W. R. Bias. "Trace Nutrient Deficiency in Daphnia magna Cultured in Standard Medium for Toxicity Testing. Effects of the Optimization of Culture Conditions on Life History Parameters of D. magna." Water Resources, Great Britain, Vol. 24, No. 9, (1990) pp 1157-1167.
2. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Mendick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
3. Field Operations Natural Resources Engineering Procedure Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.



Water Chemistry Mean Values and Ranges for *Ceriodaphnia* Tests,  
ERC Wetlands-TNT Degradation Study, September 29-October 6, 1995

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness	
	Final	(°C)	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Daphnid Control	25.0 (24.1-25.7)		7.8 (7.7-7.9)	7.5 (7.3-7.8)	8.0 (7.7-8.1)	8.0 (7.8-8.3)	332 (327-346)		63 (60-66)		91.3 (90.0-94.0)	
Aquarium 2	25.0 (24.1-25.7)		8.1 (7.3-8.4)	7.5 (7.2-7.7)	7.0 (6.6-7.7)	7.5 (7.4-7.7)	46 (37-58)		22 (17-28)		14.9 (10.0-18.0)	
Aquarium 4	25.0 (24.1-25.7)		7.9 (7.5-8.3)	7.7 (7.2-7.9)	7.9 (7.5-8.3)	8.3 (8.1-8.6)	231 (223-246)		113 (111-118)		94.0 (90.0-98.0)	
Aquarium 6	25.0 (24.1-25.7)		7.9 (7.2-8.2)	7.6 (7.3-8.0)	6.8 (6.0-7.3)	7.3 (7.2-7.4)	20 (17-22)		8 (7-9)		4.3 (2.0-6.0)	
Aquarium 10	25.0 (24.1-25.7)		8.0 (7.2-8.3)	7.7 (7.4-8.0)	7.5 (7.3-8.2)	8.1 (7.9-8.5)	182 (173-189)		89 (85-92)		75.4 (74.0-78.0)	
Aquarium 11	25.0 (24.1-25.7)		8.0 (7.8-8.4)	7.8 (7.4-8.0)	8.1 (7.7-8.3)	8.3 (8.2-8.6)	253 (250-259)		125 (122-126)		107.1 (106.0-108.0)	
Aquarium 13	25.0 (24.1-25.7)		8.0 (7.8-8.4)	7.8 (7.4-8.0)	8.0 (7.8-8.2)	8.2 (8.0-8.4)	166 (144-178)		81 (70-87)		76.6 (66.0-82.0)	
Aquarium 15	25.0 (24.1-25.7)		8.1 (7.8-8.4)	7.7 (7.5-8.0)	8.0 (7.8-8.2)	8.3 (8.1-8.5)	241 (231-245)		119 (114-121)		100.0 (96.0-102.0)	
Aquarium 16	25.0 (24.1-25.7)		7.8 (7.1-8.4)	7.7 (7.4-7.9)	6.9 (6.1-7.2)	7.4 (7.2-7.7)	18 (16-22)		8 (6-9)		4.9 (2.0-8.0)	
Aquarium 19	25.0 (24.1-25.7)		7.9 (6.2-8.4)	7.6 (7.4-7.9)	6.6 (6.0-7.1)	7.2 (7.1-7.3)	20 (18-22)		9 (8-9)		5.1 (2.0-8.0)	
Aquarium 20	25.0 (24.1-25.7)		7.9 (7.1-8.4)	7.7 (7.4-8.0)	7.5 (7.2-7.9)	8.0 (7.6-8.3)	156 (141-173)		76 (69-83)		70.0 (66.0-76.0)	

\* mg/L as CaCO<sub>3</sub>



TENNESSEE VALLEY AUTHORITY  
TOXICITY TESTING LABORATORY (TTL)  
STANDARD REPORT FORM

TOXICITY BIOMONITORING USING  
*CERIODAPHNIA DUBIA* (DAPHNIDS)

Test Title: ERC-Wetlands TNT/RDX Source Water Test, Fourth Quarter, 1995

Principal Investigator: Cynthia L. Russell

Report Preparation: Damien J. Simbeck

Starting Date: October 18, 1995

Ending Date: October 24, 1995

1.0 EXECUTIVE SUMMARY

Toxicity tests were conducted September 29-October 6, 1995 at TVA's Toxicity Testing Laboratory using outflow water samples from simulated constructed wetland aquaria to determine if chronic toxicity was present in water samples containing TNT/RDX after passing through different constructed wetlands. To help determine the cause of toxicity present in some of the samples (reported separately), a second toxicity test was conducted to determine whether toxicity was present in the source water prior to passage through the wetlands. The organism specified for testing was the daphnid (*Ceriodaphnia dubia*).

Tests conducted using a source water sample, collected October 17, 1995, demonstrated toxicity (survival and reproduction) to daphnids. Passage of water through all constructed wetlands tested in the previous study reduced toxicity, as compared to the source water.

2.0 SYSTEM OPERATION

Location/Description: The source water used for toxicity testing was collected from Well 100 at the Milan Army Ammunition Plant in Milan, TN. It was collected and transported to TVA's Wetlands Research Laboratory on August 24, 1995.



### 3.0 SAMPLE COLLECTION/TREATMENT

- 3.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The sample used for biomonitoring was a single grab sample collected from a tank at TVA's Wetlands laboratory.
- 3.2 Control and/or Dilution Water: Modified Moderately Hard Synthetic Water was used for dilution and control (see Section 4.3).
- 3.3 Test Treatments: The sample was tested at 100% only.
- 3.4 Sample Collection and Use: The source water sample was a single grab collected at 0800 CDT on October 17, 1995.
- 3.5 Sampling Method: The sample was a single grab sample collected in a pre-rinsed, new 1-gallon plastic jar. The sample was collected so that no air remained in the sample container during shipment.
- 3.6 Sample Storage/Handling: The source water sample was shipped and stored at room temperature, without aeration, to simulate the storage techniques at the Wetlands laboratory.
- 3.7 Sample Transport: The source water sample was shipped to TTL via TVA Mail.
- 3.8 Sample Pretreatment: The sample was filtered through a 64- $\mu$ m nylon mesh filter into 600-mL beakers. Sample temperature was raised to 25°C in a warm water bath and aerated as necessary to bring DO levels to near 100 percent saturation.

### 4.0 TEST ORGANISMS/CULTURE CONDITIONS

- 4.1 Species: Ceriodaphnia dubia, daphnid
- 4.2 Source: In-house culture, TVA, Toxicity Testing Laboratory
- 4.3 Culture Water: Culture medium is moderately hard synthetic water containing trace elements, macronutrients, and vitamins (modified from Elendt and Bias, 1990). [1] Water used for culture contains EDTA, while water used for test control and dilution does not.
- 4.4 Temperature of Culture: 25°C  $\pm$  1°C



- 4.5 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates from broods as described below on 2 or 3 consecutive days, 6-10 days prior to test initiation. Adults up to 14 days old may be used for neonate production. These animals are raised individually, and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass culturing may also be required at times to maintain sufficient brood stock for supporting the above. Mass cultures are maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adults, from broods containing 8 or more young.

- 4.6 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/013 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [2]

In addition to the yeast/alfalfa fish food recipe, the alga Selenastrum capricornutum concentrated to  $30 \times 10^6$  cells/mL is also fed as part of the regular diet. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal on 0.1 mL food recipe only on intermediate days.

## 5.0 TEST METHODS

- 5.1 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0 [2]

- 5.2 Modifications/Deviations to Method 1002.0:

- 5.2.1 Modified synthetic water (see Section 4.3)

- 5.2.2 Prepared Food recipe (see Section 4.6)

- 5.2.3 Feeding regime (see Section 5.11)

- 5.3 Date/Time Test Initiated: October 18, 1995/0930-1000 CDT

- 5.4 Date/Time Test Terminated: October 24, 1995/0930-0940 CDT

- 5.5 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)  
Volume per Chamber: 15 mL



- 5.6 Number of Organisms per Chamber: 1
- 5.7 Number of Replicates per Treatment: 10
- 5.8 Control/Dilution Water: Modified moderately hard synthetic water
- 5.9 Renewal period: 24-h
- 5.10 Test Temperature:  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- 5.11 Feeding Regime During Test: Each organism was fed 0.1 mL prepared food and 0.2 mL algae concentrate daily (added to renewal water before introduction of test organism).
- 5.12 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Final measurements of temperature were taken daily in 10 randomly selected cups when the test tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 7.7.
- 5.13 Statistics: Fisher's Exact Test was used to determine significant survival effects, if applicable. EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [2] No statistical analysis on sublethal (reproduction) effects could be conducted due to significant a reduction in survival based on Fisher's Exact Test.

## 6.0 QUALITY ASSURANCE

- 6.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-89/001. [2] Any known deviations were noted the study and are reported herein.



## 6.2 Physical and Chemical Methods

6.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

6.2.2 Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.

6.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [3]

6.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [3]

6.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [3]

6.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [3]

6.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N  $\text{H}_2\text{SO}_4$  to an endpoint of 4.5 according to TVA procedure ES-42.1. [3]

6.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

## 6.3 Reference Toxicant Tests

6.3.1 Test Type: Daphnids -- 7-day chronic (NOEC,  $\text{IC}_{25}$ )

6.3.2 Standard Toxicant Used: Sodium Chloride (NaCl crystalline)

6.3.3 Dilution Water Used: modified moderately hard synthetic water - daphnid chronic



- 6.3.4 Statistics: Chronic Test, NOEC - Dunnett's or Steel's Many-One Rank Tests, IC25 - EPA Bootstrap Procedure

## 7.0 RESULTS

### 7.1 Ceriodaphnia Survival and Reproduction Test

- 7.2 Summary of Results: Exposure of daphnids to a source water sample collected October 17, 1995, resulted in toxicity (survival and reproduction) to daphnids, with a 60% reduction in survival during a six day period.

### 7.3 Results, Survival Data:

Daily Percent Survival Summary for *Ceriodaphnia* Survival Test, ERC-Wetlands Source Water Test, October 18-24, 1995.

Treatment	Total Daily % Survival							S*
	Day 1	2	3	4	5	6	7	
Control	100	100	100	100	100	100	100	
Source Water	100	60	40	40	40	40	40	*

\*Significantly less than control based on Fisher's Exact Test

### 7.7 Results, Reproduction Data:

- 7.7.2 Summary of Reproduction (#young/female/6 days) data for *Ceriodaphnia* Survival Test, ERC-Wetlands Source Water Test, October 18-24, 1995.

Treatment	Reproduction (# young/female/6 days) Data										Mean	S*
	Replicate											
	1	2	3	4	5	6	7	8	9	10		
Control	31	30	32	32	31	27	30	30	30	31	30.4	
Source Water	0	0	0	0	2	0	0	1	0	0	0.3	

\*S - No statistical comparison due to significant reduction in survival.



7.8 Physical/Chemical Parameters

7.8.1 Overall Test Temperature:

Source Water Test: Mean =25.0°C (24.5°C-25.9°C)

7.8.2 Results: Water chemistry summary for ERC-Wetlands Source Water Test, October 18-24, 1995.

See: Appendix A-Water Chemistry Mean Values and Ranges for *Ceriodaphnia* Test, ERC-Wetlands Source Water Test, October 18-24, 1995.

7.9 Reference Toxicant Tests

7.9.1 Summary of Results:

The most recent reference toxicant test conducted prior ERC-Wetlands TNT Degradation Study, September 29-October 6, 1995, showed chronic results consistent with TTL control chart ranges.

7.9.2 *Ceriodaphnia*

7.9.2.1 Chronic Tests

7.9.2.2 Date/Time of Most Recent Test: September 27-October 3, 1995/1010 CDT

7.9.2.3 NOEC: 900 mg NaCl/L  
LOEC: 1300 mg NaCl/L  
IC25: 1354 mg NaCl/L

7.9.2.4 Control Chart Information:

Number of standard tests completed by laboratory: 21

NOEC Range: 490-1300 mg NaCl/L  
LOEC Range: 900-1800 mg NaCl/L  
IC25 Range: 921-1574 mg NaCl/L (mean  $\pm$  two standard deviations)

8.0 CONCLUSION

Tests conducted using a source water sample, collected October 17, 1995, demonstrated toxicity (survival and reproduction) to daphnids. Passage of water through all constructed wetlands tested in the previous study reduced toxicity, as compared to the source water.



1. Elendt, B. P., and W. R. Bias. "Trace Nutrient Deficiency in Daphnia magna Cultured in Standard Medium for Toxicity Testing. Effects of the Optimization of Culture Conditions on Life History Parameters of D. magna." Water Resources, Great Britain, Vol. 24, No. 9, (1990) pp 1157-1167.
2. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Mendick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
3. Field Operations Natural Resources Engineering Procedure Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.



# Appendix A

## Water Chemistry Mean Values and Ranges for *Ceriodaphnia* Tests, ERC Wetlands Source Water Test, October 18-24, 1995

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness	
	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial
	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(S.U.)	(µmhos)	(64-64)	(90.0-90.0)		*
Daphnid Control	25.0 (24.5-25.9)	8.1 (7.9-8.4)	7.9 (7.4-8.1)	8.3 (8.2-8.3)	8.1 (8.0-8.2)	330 (328-331)			64 (64-64)	90.0 (90.0-90.0)		
Source Water	25.0 (24.5-25.9)	8.3 (8.1-8.4)	8.0 (7.7-8.2)	6.4 (6.1-6.6)	7.5 (7.3-7.6)	111 (106-125)			5 (5-5)	26.0 (26.0-26.0)		

\* mg/L as CaCO<sub>3</sub>



December 21, 1995

Steven H. Coonrod, WET 1A-M  
Frank J. Sikora, CEB 1C-M

ASSESSING SEDIMENT QUALITY IN RELATION TO METALS AND PAH BIOAVAILABILITY  
IN JACOB'S CREEK AT THE PARADISE FOSSIL PLANT - REPORT OF AUGUST 1995  
TOXICITY/METALS PARTITIONING RESULTS

Attached is the subject report that describes the Jacob's Creek sediment toxicity evaluation of EPA's metals partitioning "no effects" criteria. Biological study methods, results, and conclusions are incorporated into the report as they relate to the AVS/SEM partitioning results obtained from the August 1995 survey. This report should be appended to the project detailed technical report referenced in Harold Speidel's October 31, 1995, memorandum to John W. Myers.

As discussed during our October 30 meeting, we should publish this information in the Environmental Toxicology and Chemistry International Journal, which is published monthly by that society (SETAC). This information is important to EPA's Contaminated Sediment Management Strategy (Draft), which incorporates both sediment toxicity tests used in this evaluation and also the AVS/SEM metals partitioning and interstitial water assessments of bioavailability (no effects criteria). During this year's Second SETAC World Congress, held in Vancouver, British Columbia, several EPA researchers asked me for a copy of our results. I feel we should provide EPA a copy of TVA's technical report.

By copy of this memorandum, I am providing this report to the Paradise Fossil Plant and to F&HP staff for their use. If you have any questions, please call me at extension 2068 in Muscle Shoals.



Donald C. Wade  
Senior Toxicologist  
Water Management  
OSA 1B-M

DCW

cc (Attachment):

A. R. Lewis, LP 5D-C  
J. W. Myers, LP 5D-C (No attachment)  
G. Quintero, CC 1A-C  
R. J. Pryor, WT 10D-K  
D. W. Robinson, LP 5H-C  
D. J. Simbeck, TTL 1A-BFN  
H. K. Speidel, CEB 3A-M (No attachment)  
R. K. Tapp, PAF 1A-DRK  
Files, WM, CST 16D-C



## STANDARD REPORT FORM

### TOXICITY BIOMONITORING *HYALELLA AZTECA* (AMPHIPOD), AND *CHIRONOMUS TENTANS* (MIDGE)

Test Title: Jacob's Creek Sediment Toxicity Evaluation of EPA's Metals Partitioning AVS/SEM  
"No Effects" Criteria, Paradise Fossil Plant

Principal Investigator: Damien J. Simbeck

Report Prepared: Donald C. Wade and Damien J. Simbeck

Starting Date: August 4, 1995

Ending Date: August 14, 1995

#### 1.0 EXECUTIVE SUMMARY

Current Environmental Protection Agency (EPA) studies are pursuing the development and use of sediment quality criteria under authority of the Clean Water Act to protect the chemical, physical, and biological integrity of the country's water resources. These criteria are intended to be used to prevent clean sediments from becoming contaminated and to assist in making regulatory and remediation decisions on sediments that are already contaminated. Sediment quality criteria are the EPA's best recommendation of the concentrations of substances in sediment that will not unacceptably affect benthic organisms. [1]

This study was conducted to evaluate applicability of EPA's "no effects" sediment quality criteria for metals at the Tennessee Valley Authority's (TVA) Paradise Fossil Plant (PAF), Drakesboro, Kentucky. The study was designed to determine amounts of toxic metals present in sediments of Jacob's Creek, a tributary stream to the Green River that receives waste water discharges from the plant's ash pond, and determine if those metals were bioavailable [2] Bioavailability was determined through chemical analyses, based on partitioning of toxic metals (Ni, Zn, Pb, Cu, and Cd) with acid volatile sulfides (AVS), measurements of toxic metal concentrations in sediment interstitial water, and also biologically, based on acute whole-sediment toxicity tests.

This report concludes the biological portion of the study. Two EPA sediment test species, an amphipod, *Hyalella azteca* and a midge, *Chironomus tentans* were exposed to sediments collected from Jacob's Creek at one station upstream from the PAF ashpond discharge and three stations downstream from the ash pond's discharge. Sediment toxicity was evaluated based on lethal and sub-lethal (growth) responses of these organisms.

Ten day exposure to Jacob's Creek sediments resulted in no survival effects to either test organism when compared to sediment at the upstream station or a control sediment formulated as prescribed in EPA sediment test methods. [3] Amphipod growth (dry weight) was statistically reduced in sediments from Stations 3 and 4 downstream from the ashpond discharge compared to both the formulated control sediment (MSD=12.3%) and sediment collected at Station 2, upstream from the ashpond discharge (MSD=11.3%). However, amphipod growth reductions were, in all cases, less than 25 percent and the reduction at station 4 compared with the upstream station was slightly less than the MSD, though



declared significant by the Dunnett's statistical test. Midge growth was statistically reduced at all stations compared to the formulated sediment control; however, there was no reduction in midge growth at downstream stations compared to growth at the upstream station. The comparisons of midge growth based on EPA's formulated control sediment should be discounted based on recent evaluations of midge growth responses using EPA's formulated sediment, where up to 40 percent of the tissue mass of larvae in fine grained (<150  $\mu$ m) sediments was actually due to ingestion of sediment particles. [4] The error in growth using the EPA formulated sediment likely ranges from 40 to 60 percent. [5]

The conclusion from the biological assessment is that Jacob's Creek sediments were not toxic to either test organism based on 10-day survival responses. Statistically significant sub-lethal (growth) reductions at the first two stations (3 and 4) downstream from the ashpond discharge were based on relatively small minimum significant differences (11-12%) for the amphipod test. SEM/AVS ratios at these stations were less than 1.0 (0.46 and 0.05) [6] and indicated that metal toxicity should not be present at these stations. Measured interstitial water total metals concentrations (0.07 mg/L and 0.01 mg/L, respectively at Stations 3 and 4) were well below toxic levels for the amphipod, *Hyalella azteca*, based on Ni and Zn, the only toxic metals detected in interstitial water analyses. Growth reductions in the midge test based on comparisons with formulated sediment appeared to be related to small particle sizes in the formulated control sediment rather than to toxic substances in the test sediments. Comparisons of midge growth based on the upstream station did not indicate downstream toxicity. These data support the use of EPA's SEM/AVS partitioning model as a "no effects" predictor of toxicity in sediments. These data indicate that toxic metals in Jacob's Creek sediments were not bioavailable and that Jacob's Creek sediments were acceptable with regards to EPA's "no effects" metals criteria during the period evaluated.

## 2.0 SAMPLE COLLECTION/TREATMENT

- 2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): Sediment samples collected from Jacob's Creek upstream and downstream of the Paradise Fossil Plant ashpond discharge were collected for chemical analyses and biological (toxicity) evaluation. Samples from four stations were collected. One station (Station 2) was upstream of the Paradise Fossil Plant ashpond discharge. The remaining three were downstream.
- 2.2 Bioassay Tests:
  - 2.2.1 Amphipod: The amphipod test was a single test conducted with a formulated sediment (negative) control and Station 2 (reference control).
  - 2.2.2 Midge: The midge test was a single test conducted with a formulated sediment (negative) control and Station 2 (reference control).
- 2.3 Control/Overlying and Dilution Water: Synthetic water with dechlorinated tap water (1:1 v:v) was used for dilution and control/overlying water for the amphipod and midge tests.
- 2.4 Control Sediment: Formulated sediment (80% clay, 20% Sand; 2% TOC) was used for the negative control sediment for the whole sediment. Station 2 was used as a site upstream sediment for evaluating downstream effects.



- 2.5 Sample Dates and Times: All sediment samples were collected on July 31, 1995. Collection times were: Station 2-0820 CDT; Station 3-0725 CDT; Station 4-1145 CDT; Station 5-1445 CDT.
- 2.6 Sampling Method: Sediment samples were collected using a core sampler with a stainless steel insert. Samples were placed into 1-L glass jars with a lined lid. A thin layer of site water was placed over the sediment and stripped with N<sub>2</sub> to maintain anoxic conditions during shipment.
- 2.7 Sample Storage/Handling: Samples were placed in coolers on ice to maintain temperatures <4°C during shipment. At TVA's Toxicity Testing Laboratory (TTL), Athens, AL, samples were stored in a refrigerator at 4°C±1°C until used in toxicity tests.
- 2.8 Sample Transport: Samples were transported to TTL by collectors the day after collection.
- 2.9 Sample Pretreatment: On August 1, all samples (9 1-L jars) from each station were compiled in a stainless steel bowl and mixed until homogenous with a stainless steel spoon. One liter of sample from each station was shipped to TVA's Environmental Chemistry Laboratory (ECHE) in Chattanooga, TN, for whole sediment chemical analyses. One liter was centrifuged at 3800 rpm for 30 minutes for porewater extraction. The porewater was then centrifuged at 10,000 rpm for 10 minutes, then 64 µm filtered for porewater ammonia analyses. The remaining 7 L was placed in separate 8-L Nalgene® jars and stored in a refrigerator until August 3. On August 3 (Test Day -1), samples were re-homogenized and then placed into the test system (100 mL/replicate) and overlying water was added.
- 2.10 Test Treatments: Each sediment sample was tested at 100 percent (undiluted) for both species.

### 3.0 TEST ORGANISMS/CULTURE CONDITIONS

#### 3.1 *Hyalella azteca*, amphipod

Amphipods were purchased from Cosper Environmental Services, Inc., Bohemia, NY, for use in whole sediment toxicity tests. Animals of correct test age (7-14 days old) were obtained by sieving. Amphipods which pass through a US Standard #35 (500 µm) sieve, but are stopped by a #45 (355 µm) sieve are the mean size of 6-d old amphipods [3]. Amphipods were shipped via priority overnight freight, and used for toxicity tests two days later (8-d old).

#### 3.2 *Chironomus tentans*, midge

##### 3.2.1 Source: In-house culture, TVA, Toxicity Testing Laboratory

##### 3.2.2 Culture Water: Culture water consists of moderately hard synthetic water mixed with dechlorinated tap water (1:1 v:v). Reagents for synthetic water were added to Milli-Q UF product water. Culture water was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 110 mg/L as CaCO<sub>3</sub>.

##### 3.2.3 Temperature of Culture: 23°C ± 2°C



3.2.4 General Maintenance: Larval midges (0-14 days old) are reared in 1.5-L glass aquaria with 1 L water and 30 mL sand. Water is renewed three times/week. When larvae are 7-14 days old, they are transferred to a 20-L glass aquaria containing 15 L water and 100 mL sand. Aquaria are placed into a flow-through recirculating system. Flow rate to aquaria is approximately one-half aquarium (10-L) per hour. Approximately 50 percent of the system water is replaced weekly. All tanks are fed daily a fish flake food or *Chlorella* suspension. Adults that emerge from the aquaria are placed into 8-L glass aquaria with a screened top and approximately 500 mL water for egg deposition. A 5x20-cm piece of screen is placed into the aquaria to provide a resting area for the adults. Egg cases are collected daily and placed into the 1.5-L aquaria with 1 L water and 30 mL sand.

3.2.5 Egg Cases Collected: July 18-20, 1995

3.2.6 Age at test initiation: 9-11 days (2<sup>nd</sup> and 3<sup>rd</sup> instar)

3.2.7 Average Head Capsule Width: 0.38 mm (0.32-0.44 mm range)

#### 4.0 TEST METHODS

4.1 Amphipod, *Hyalella azteca*, Survival Test, EPA Test Method 100.1 [3]

4.1.1 Modifications/Deviations to 100.1: Water feed rates varied more than 10% among replicates using the syringe system during system calibration. Test beakers were therefore rotated daily in the system so that all replicates would receive water from each syringe during the test.

4.1.2 Date/Time Test Initiated: August 4, 1995/0905-0900 CDT

4.1.3 Date/Time Test Terminated: August 14, 1995/0815-1145 CDT

4.1.4 Age of Test Organism: 7-14 day

4.1.5 Test Chamber: 400 mL beaker with 350 µm Nitex® mesh covering a notch at the top

4.1.6 Volume in Chamber: 100 mL sediment, 175 mL water

4.1.7 Number of Organisms per Replicate: 10

4.1.8 Number of Replicates per Treatment: 8

4.1.9 Test Control Sediment: Formulated Sediment (Negative Control)  
Station 2 (Reference Control)

4.1.10 Dilution Sediment: NA

4.1.11 Overlying Water: Moderately hard synthetic water plus dechlorinated tap water, mixed 1:1 (v:v).

4.1.12 Dilution water: Same



- 4.1.13 Test Temperature:  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (Instantaneous temperature  $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ )
- 4.1.14 Photoperiod: 16 L:8D
- 4.1.15 Renewal Period: Overlying Water renewed twice daily by slow flow delivery system.
- 4.1.16 Renewal Method: Water was fed from a head tank with eight 10-mL syringes so that it would flow slowly (about 200 mL/10 minutes) into each test chamber.
- 4.1.17 Feeding Regime During Test: 1.5 mL/replicate YCT once daily
- 4.1.18 Physical and Chemical Parameters Measured: Parameters measured on new batches of medium ("initial") were temperature (adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, and hardness.  
  
"Final" measurements of temperature, was taken in one replicate per treatment before renewal. "Final" measurements of DO, pH and conductivity were taken daily and alkalinity and hardness were taken on Days 1, 5 and 10 in a combination of about 75 mL samples removed daily from all replicates per treatment before renewal. On Days 1, 5 and 10, test solutions (100 mL) were preserved with 1:4  $\text{H}_2\text{SO}_4$  and refrigerated until sent to TVA's Environmental Chemistry Laboratory in Chattanooga Tennessee, for ammonia analysis using the automated alkaline phenate methodology.
- 4.1.19 Test Endpoint Determination:
  - 4.1.19.1 Survival: Test animals were counted as dead if they could not be found at test termination
  - 4.1.19.2 Growth: Growth was determined by dry weight measurements after test termination.
- 4.1.20 Statistics: Revised statistical procedures contained in the fourth edition of EPA's acute toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [7] The tests used to determine toxicity endpoints were Steel's Many-One Rank Test for survival data and Dunnett's Test for growth data. Statistical analyses were conducted using both formulated sediment and station 2 as control.
- 4.2 Midge, *Chironomus tentans*, EPA Test Method 100.2 [3]
  - 4.2.1 Modifications/Deviations to 100.1: Water feed rates varied more than 10% among replicates using the syringe system during system calibration. Test beakers were therefore rotated daily in the system so that all replicates would receive water from each syringe during the test.
  - 4.2.2 Date/Time Test Initiated: August 4, 1995/1045-1100 CDT
  - 4.2.3 Date/Time Test Terminated: August 14, 1995/0815-1245 CDT
  - 4.2.4 Age of Test Organism: 9-11 days (2<sup>nd</sup> and 3<sup>rd</sup> instar)



- 4.2.5 Test Chamber: 400 mL beaker with 350  $\mu$ m Nitex® mesh covering a notch at the top
- 4.2.6 Volume in Chamber: 100 mL sediment, 175 mL water
- 4.2.7 Number of Organisms per Replicate: 10
- 4.2.8 Number of Replicates per Treatment: 8
- 4.2.9 Test Control Sediment: Formulated Sediment (Negative Sediment)  
Station 2 (Reference Sediment)
- 4.2.10 Dilution Sediment: NA
- 4.2.11 Overlying Water: Moderately hard synthetic water plus dechlorinated tap water, mixed 1:1 (v:v).
- 4.2.12 Dilution water: NA
- 4.2.13 Test Temperature:  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (Instantaneous temperature  $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ )
- 4.2.14 Photoperiod: 16 L:8D
- 4.2.15 Renewal Period: Overlying Water renewed twice daily by slow flow delivery system.
- 4.2.16 Renewal Method: Water was fed from a head tank with eight 10-mL syringes so that it would flow slowly (about 200 mL/10 minutes) into each test chamber.
- 4.2.17 Feeding Regime During Test: 1.5 mL/replicate fish flake food suspension (containing 2.7g/L flakes) once daily. Feeding was suspended when DO levels were below 5.5 mg/L for two consecutive days in one treatments, or on one day in two or more treatments.
- 4.2.18 Physical and Chemical Parameters Measured: Parameters measured on new batches of medium ("initial") were temperature (adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, and hardness.
- "Final" measurements of temperature, was taken in one replicate per treatment before renewal. "Final" measurements of DO, pH and conductivity were taken daily and alkalinity and hardness were taken on Days 1, 5 and 10 in a combination of about 75 mL samples removed daily from all replicates per treatment before renewal. On Days 1, 5 and 10, test solutions (100 mL) were preserved with 1:4  $\text{H}_2\text{SO}_4$  and refrigerated until sent to TVA's Environmental Chemistry Laboratory in Chattanooga Tennessee, for ammonia analysis using the automated alkaline phenate methodology.
- 4.2.19 Test Endpoint Determination:
- 4.2.19.1 Survival: Test animals were counted as dead if they could not be found at test termination



- 4.2.19.2 Growth: Growth was determined by dry weight measurements after test termination.
- 4.2.20 Statistics: Revised statistical procedures contained in the fourth edition of EPA's acute toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [7] The statistical test used to determine toxicity endpoints was Dunnett's Test for both survival and growth data. Statistical analyses were conducted using both formulated sediment and station 2 as control.

## 5.0 QUALITY ASSURANCE

- 5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-89/001. [8][7] Any known deviations were noted the study and are reported herein.
- 5.2 Physical and Chemical Methods
- 5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.
- 5.2.2 Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.
- 5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [9]
- 5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [9]
- 5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [9]
- 5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [9]
- 5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H<sub>2</sub>SO<sub>4</sub> to an endpoint of 4.5 according to TVA procedure ES-42.1. [9]
- 5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colormetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.



5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [9]

### 5.3 Reference Toxicant Tests

5.3.1 Test Type: Amphipod -- 96-hr acute, water only (LC<sub>50</sub>)  
Midge -- 96-hr acute, water only (LC<sub>50</sub>)

5.3.2 Standard Toxicant Used: Potassium Chloride

5.3.3 Dilution Water Used: Moderately hard synthetic water with 50% dechlorinated tap water

5.3.4 Statistics: Probit's, Spearman-Kärber, etc.

## 6.0 RESULTS

### 6.1 Summary of Bioassay Results:

#### 6.2 Amphipod, *Hyalella azteca*

6.2.1 Summary of Results: Ten-day exposure of amphipods to the Jacob's Creek sediments resulted in no survival effects. Tests showed a significant decrease in growth to amphipods in sediments from Stations 3 and 4, although the decrease at Station 4 was below the MSD when compared to the upstream site.

6.2.2 Analysis of Survival Data Using Steel's Many-One Rank Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Formulated Sediment Control**

Treatment	Amphipod Survival Data (% Survival)								Mean	S*
	Replicate									
	1	2	3	4	5	6	7	8		
Formulated Sediment	100	90	100	90	90	100	100	90	95	
Station 2	100	100	90	90	80	100	100	100	95	
Station 3	100	100	90	100	80	80	80	100	91	
Station 4	100	100	100	100	100	100	90	100	99	
Station 5	100	90	100	100	100	100	100	80	96	

Treatment	Steel's Many-One Rank Test		
	Number of Replicates	Critical Rank Sum	Rank Sum*
Station 2	8	47	70
Station 3	8	47	62
Station 4	8	47	80
Station 5	8	47	74

\*Values less than Critical Rank Sum significantly less than control (Formulated Sediment)



## 6.2.3

Analysis of Survival Data Using Steel's Many-One Rank Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Station 2, Upstream Sediment Comparison**

Treatment	Amphipod Survival Data (% Survival)								Mean	S*
	Replicate									
	1	2	3	4	5	6	7	8		
Station 2	100	100	90	90	80	100	100	100	95	
Station 3	100	100	90	100	80	80	80	100	91	
Station 4	100	100	100	100	100	100	90	100	99	
Station 5	100	90	100	100	100	100	100	80	96	

Steel's Many-One Rank Test			
Treatment	Number of Replicates	Critical Rank Sum	Rank Sum*
Station 3	8	48	61.5
Station 4	8	48	76.5
Station 5	8	48	71.5

\*Values less than Critical Rank Sum significantly less than control (Station 2)

## 6.2.4

Analysis of Growth (Dry Weight [mg]) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Formulated Sediment Control**

No transformation applied before data analysis.

For this set of data, the minimum significant difference is 0.02 mg.

This represents a 12.30% reduction in Dry Weight (mg)

T = 2.24

Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data)										
Treatment	Replicate								Mean	S*
	1	2	3	4	5	6	7	8		
Formulated Sediment	0.189	0.171	0.127	0.156	0.139	0.157	0.174	0.130	0.155	
Station 2	0.169	0.163	0.148	0.131	0.125	0.149	0.178	0.150	0.152	
Station 3	0.121	0.100	0.120	0.120	0.154	0.118	0.111	0.129	0.122	*
Station 4	0.153	0.122	0.141	0.125	0.153	0.142	0.130	0.117	0.135	*
Station 5	0.148	0.190	0.159	0.125	0.160	0.144	0.136	0.140	0.150	

Asterisk (\*) indicates values significantly less than control (Formulated Sediment).



6.2.5 Analysis of Growth (Dry Weight [mg]) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Station 2, Upstream Sediment Comparison**

No transformation applied before data analysis.

For this set of data, the minimum significant difference is 0.02 mg.

This represents a 11.30% reduction in Dry Weight (mg)

T = 2.16

Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Treatment	Replicate								Mean	S*
	1	2	3	4	5	6	7	8		
Station 2	0.169	0.163	0.148	0.131	0.125	0.149	0.178	0.150	0.152	
Station 3	0.121	0.100	0.120	0.120	0.154	0.118	0.111	0.129	0.122	*
Station 4	0.153	0.122	0.141	0.125	0.153	0.142	0.130	0.117	0.135	*†
Station 5	0.148	0.190	0.159	0.125	0.160	0.144	0.136	0.140	0.150	

Asterisk (\*) indicates values significantly less than control (Station 2).

†Growth reduction less than the MSD.

6.3 Midge, *Chironomus tentans*

6.3.1 Summary of Results: Ten-day exposure of midges to sediments from Jacob's Creek resulted in no survival effects. A significant reduction in growth occurred at all stations, including the upstream site, when compared to the negative (Formulated Sediment) control. No significant reduction in growth was seen in any station when compared to the upstream control.

6.3.2 Analysis of Survival (%) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Formulated Sediment Control**

Arcsine square-root transformation applied before data analysis.

For this set of data, the minimum significant difference is 22.

This represents a 30.82% reduction in Survival (%)

T = 2.24

Alpha = 0.05 (one-tailed test)

Midge Survival Data (% Survival)

Treatment	Replicate								Mean	S*
	1	2	3	4	5	6	7	8		
Formulated Sediment	80	100	40	40	70	80	70	80	70	
Station 2	80	90	60	100	100	70	70	60	79	
Station 3	80	90	50	70	80	60	40	70	68	
Station 4	90	60	60	60	80	60	70	90	71	
Station 5	60	90	80	50	40	60	70	90	68	

Asterisk (\*) indicates values significantly less than control (Formulated Sediment)



## 6.3.3

Analysis of Survival (%) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Station 2, Upstream Sediment Comparison**

Arcsine square-root transformation applied before data analysis.  
For this set of data, the minimum significant difference is 19.

This represents a 23.19% reduction in Survival (%)

$$T = 2.16$$

$$\text{Alpha} = 0.05 \text{ (one-tailed test)}$$

Treatment	Midge Survival Data (% Survival)								Mean	S*
	Replicate									
	1	2	3	4	5	6	7	8		
Station 2	80	90	60	100	100	70	70	60	79	
Station 3	80	90	50	70	80	60	40	70	68	
Station 4	90	60	60	60	80	60	70	90	71	
Station 5	60	90	80	50	40	60	70	90	68	

Asterisk (\*) indicates values significantly less than control (Station 2)

## 6.3.4

Analysis of Growth (Dry Weight [mg]) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Formulated Sediment Control**

No transformation applied before data analysis.  
For this set of data, the minimum significant difference is 0.22 mg.

This represents a 15.76% reduction in Dry Weight (mg)

$$T = 2.24$$

$$\text{Alpha} = 0.05 \text{ (one-tailed test)}$$

Treatment	Midge Dry Weight (mg)								Mean	S*
	Replicate									
	1	2	3	4	5	6	7	8		
Formulated Sediment	1.044	1.247	1.732	1.475	1.563	1.115	1.673	1.493	1.418	
Station 2	0.933	0.560	0.812	0.745	0.664	0.654	0.691	0.842	0.738	*
Station 3	0.803	0.938	0.754	0.877	0.580	0.672	1.298	0.734	0.832	*
Station 4	0.597	1.068	0.725	0.755	0.745	0.740	0.876	0.833	0.792	*
Station 5	0.833	0.732	0.896	1.296	1.220	1.012	0.784	0.670	0.931	*

Asterisk (\*) indicates values significantly less than control (Formulated Sediment)



6.3.5 Analysis of Growth (Dry Weight [mg]) Data Using Dunnett's Test, Jacob's Creek Sediment Test, August 4-14, 1995. **Station 2, Upstream Sediment Comparison**

No transformation applied before data analysis.

For this set of data, the minimum significant difference is 0.20 mg.

This represents a 26.91% reduction in Dry Weight (mg)

T = 2.16

Alpha = 0.05 (one-tailed test)

Treatment	Midge Dry Weight (mg)								Mean	S*
	Replicate									
	1	2	3	4	5	6	7	8		
Station 2	0.933	0.560	0.812	0.745	0.664	0.654	0.691	0.842	0.738	
Station 3	0.803	0.938	0.754	0.877	0.580	0.672	1.298	0.734	0.832	
Station 4	0.597	1.068	0.725	0.755	0.745	0.740	0.876	0.833	0.792	
Station 5	0.833	0.732	0.896	1.296	1.220	1.012	0.784	0.670	0.931	

Asterisk (\*) indicates values significantly less than control (Station 2)

6.5 Physical/Chemical Parameters

6.5.1 Overall Test Temperature:

6.5.2 Amphipod: Mean = 23.1°C (22.7°C-23.3°C)

6.5.4 Midge: Mean = 22.9°C (22.4°C-23.3°C)

6.6 Results: Water chemistry and sediment analysis summaries for Jacob's Creek Sediment Bioassay, August 4-14, 1995.

See: Appendix A Water Chemistry Mean Values and Ranges for Jacob's Creek Sediment Bioassay, August 4-14, 1995

See: Appendix B Toxicity Split-Sample Sediment Analysis, Metals, Pesticides and Solids, Jacob's Creek Sediment Bioassay, August 4-14, 1995

See: Appendix C Sediment Metals Partitioning, Jacob's Creek, August 1995

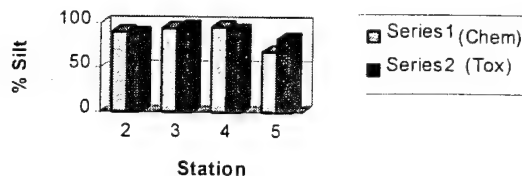
6.6.1 Of the five toxic metals (Cu, Pb, Ni, Zn, and Cd) included in EPA's "no effects" AVS/SEM partitioning criteria, Ni and Zn were the predominant toxic metals in the Jacob's Creek whole-sediment samples. As illustrated in Section 6.6.2 and Section 6.2.3, there was very little difference between sediment samples split from toxicity samples and sediment samples analyzed for metals partitioning based on particle size and whole sediment metals analysis when the data were normalized for percent moisture and expressed in molar units. These data predict close agreement between toxicological and partitioning results.



## 6.6.2

Comparison of Silt Content in Sediment Samples Analyzed for Metals Partitioning (Chem) and Sediment Samples Split from Toxicity Samples (Tox)

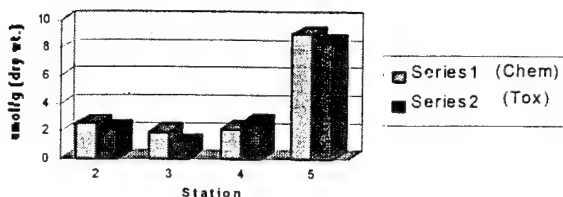
Sediment Percent Silt, Jacob's Creek, August, 1995



## 6.6.3

Comparison of Toxic Metals in Sediments Analyzed for Metals Partitioning (Chem) and Toxic Metals in Sediments Split from Toxicity Samples (Tox)

Sediment Toxic Metals, Jacob's Creek, August 1995



## 6.7

Reference Toxicant Tests

## 6.7.1

Summary of Results:

The most recent midge reference toxicants tests conducted prior Jacob's Creek Sediment Bioassay showed acute results consistent with TTL control chart ranges. Reference toxicant tests conducted using amphipods initiated one day prior to this study showed acute results higher than prior tests at TTL as well as those published in EPA 600/R-94/024. [3]

## 6.7.2

Amphipods

## 6.7.2.1

Date/Time of Most Recent Test: August 3-7, 1995/1230 CDT

## 6.7.2.2

LC<sub>50</sub>: 413 mg/L KCl

## 6.7.2.3

TTL Test Summary:

Number of standard tests completed by laboratory: 4

LC<sub>50</sub> Range: 274-413 mg/L KCl



6.7.2.4 EPA Test Summary:

Number of laboratories conducting tests: 9

LC<sub>50</sub> Mean and Ranges: 305.0 mg/L KCl (232.0-372.0 mg/L KCl)

6.7.3 Midges

6.7.3.1 Date/Time of Most Recent Test: August 3-7, 1995/1215 CDT

6.7.3.2 LC<sub>50</sub>: 6234 mg/L KCl

6.7.3.3 Control Chart Information:

Number of standard tests completed by laboratory: 6

LC<sub>50</sub> Range: 4836-6786 mg/L KCl (Mean  $\pm$  2 standard deviations)

7.0 CONCLUSIONS

7.1 Two EPA sediment test species, an amphipod, *Hyalella azteca* and a midge, *Chironomus tentans* were exposed to sediments collected from Jacob's Creek at one station upstream from the PAF ashpond discharge and three stations downstream from the ash pond's discharge. Sediment toxicity was evaluated based on lethal and sub-lethal (growth) responses of these organisms.

Ten day exposure to Jacob's Creek sediments resulted in no survival effects to either test organism when compared to sediment at the upstream station or a control sediment formulated as prescribed in EPA sediment test methods. [3] Amphipod growth (dry weight) was statistically reduced in sediments from Stations 3 and 4 downstream from the ashpond discharge compared to both the formulated control sediment (MSD=12.3%) and sediment collected at Station 2, upstream from the ashpond discharge (MSD=11.3%). However, amphipod growth reductions were, in all cases, less than 25 percent and the reduction at station 4 compared with the upstream station was slightly less than the MSD, though declared significant by the Dunnett's statistical test. Midge growth was statistically reduced at all stations compared to the formulated sediment control; however, there was no reduction in midge growth at downstream stations compared to growth at the upstream station. The comparisons of midge growth based on EPA's formulated control sediment should be discounted based on recent evaluations of midge growth responses using EPA's formulated sediment, where up to 40 percent of the tissue mass of larvae in fine grained (<150  $\mu$ m) sediments was actually due to ingestion of sediment particles. [4] The error in growth using the EPA formulated sediment likely ranges from 40 to 60 percent. [5]

The conclusion from the biological assessment is that Jacob's Creek sediments were not toxic to either test organism based on 10-day survival responses. Statistically significant sub-lethal (growth) reductions at the first two stations (3 and 4) downstream from the ashpond discharge were based on relatively small minimum significant differences (11-12%) for the amphipod test. SEM/AVS ratios at these stations were less than 1.0 (0.46 and 0.05) [6] and indicated that metal toxicity should not be present at these stations. Measured interstitial water total



metals concentrations (0.07 mg/L and 0.01 mg/L, respectively at Stations 3 and 4) were well below toxic levels for the amphipod, *Hyalella azteca*, based on Ni and Zn, the only toxic metals detected in interstitial water analyses (Appendix C). Growth reductions in the midge test based on comparisons with formulated sediment appeared to be related to small particle sizes in the formulated control sediment rather than to toxic substances in the test sediments. Comparisons of midge growth based on the upstream station did not indicate downstream toxicity. These data support the use of EPA's SEM/AVS partitioning model as a "no effects" predictor of toxicity in sediments. These data indicate that toxic metals in Jacob's Creek sediments were not bioavailable and that Jacob's Creek sediments were acceptable with regards to EPA's "no effects" metals criteria during the period evaluated.

## 8.0 REFERENCES

1. U. S. EPA. "Briefing Report to the EPA Science Advisory Board on the Equilibrium Partitioning Approach to Predicting Metal Bioavailability in Sediments and the Derivation of Sediment Quality Criteria for Metals." EPA-822-D-94-002. Office of Water and Office of Research and Development. December 1994.
2. Project Plan, Assessing Sediment Quality in Relation to Metals and PAH Bioavailability in Jacob's Creek at the Paradise Fossil Plant, TVA, February, 1995.
3. U. S. EPA, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, EPA 600/R-94/024 (June 1994)
4. Sibley, P. K., D. A. Benoit, and G. T. Ankley. "Particle Size as a Modifying Factor in Sediment Toxicity Tests: Effects on Growth, Reproduction, and Behavior in *Chironomus tentans*." Poster PW183 presented at the Second SETAC World Congress, Vancouver, BC, Canada, November 5-9, 1995 (page 263 of the published abstracts).
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6. TVA. "Assessing Sediment Quality in Relation to Metals and PAH Bioavailability in Jacob's Creek at the Paradise Fossil Plant," Project Number 95177 Closure Report, TVA Research and Technology Department Projects, Environmental Research Center. October 30, 1995.
7. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Mendick, T. W. Neihsel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
8. Toxicity Testing Laboratory Quality Assurance Program and Standard Operating Procedures Manual, Division of Water Resources, Tennessee Valley Authority (October 1992).



9. Field Operations Natural Resources Engineering Procedure Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.



# Appendix A

## Water Chemistry Mean Values and Ranges for Jacob's Creek Sediment Test, August 4-14, 1995

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness		Ammonia		Chlorine	
	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial
Overlying Water	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	*	*	*	(mg/L)	(mg/L)	(mg/L)	(mg/L)
	-	8.3 (8.1-8.4)	-	8.2 (8.2-8.2)	-	305 (304-306)	-	77 (72-81)	-	107.0 (102.0-112.0)	-	-	-	-	-	<0.1 (<0.1-<0.1)
Form. Sediment <i>H. azteca</i>	23.2 (22.8-23.9)	-	6.1 (5.3-7.0)	-	7.6 (7.5-7.7)	-	353 (333-418)	-	87 (85-90)	-	122.7 (118.0-128.0)	<0.002 (<0.001-0.002)	-	-	-	-
Station 2 <i>H. azteca</i>	23.0 (22.8-23.6)	-	6.1 (5.6-6.5)	-	8.1 (7.9-8.5)	-	379 (363-420)	-	106 (98-113)	-	134.7 (128.0-142.0)	0.084 (0.068-0.110)	-	-	-	-
Station 3 <i>H. azteca</i>	23.1 (22.7-23.7)	-	6.0 (5.6-6.2)	-	7.5 (7.3-7.6)	-	315 (307-324)	-	68 (60-77)	-	108.7 (106.0-110.0)	0.012 (0.002-0.021)	-	-	-	-
Station 4 <i>H. azteca</i>	23.1 (22.9-23.7)	-	6.0 (5.7-6.3)	-	7.6 (7.5-7.6)	-	329 (318-350)	-	78 (75-80)	-	112.7 (110.0-114.0)	0.025 (0.001-0.048)	-	-	-	-
Station 5 <i>H. azteca</i>	23.3 (23.1-23.9)	-	6.1 (5.8-6.4)	-	7.7 (7.6-7.9)	-	344 (331-374)	-	85 (81-89)	-	125.3 (120.0-134.0)	0.020 (0.002-0.053)	-	-	-	-

\* mg/L as CaCO<sub>3</sub>



# Appendix A (Continued)

## Water Chemistry Mean Values and Ranges for Jacob's Creek Sediment Test, August 4-14, 1995

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alkalinity		Hardness		Ammonia		Chlorine	
	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial
	(°C)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	*	*	*	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Form. Sediment	22.9	-	5.5	-	7.5	-	366	-	90	-	129.3	-	<0.001	<0.001	-	-
<i>C. tentans</i>	(22.6-23.6)		(3.8-7.3)		(7.3-7.7)		(348-411)		(86-97)		(124.0-134.0)		(<0.001-<0.001)			
Station 2	22.8	-	6.4	-	7.9	-	365	-	99	-	129.3	-	0.060	0.060	-	-
<i>C. tentans</i>	(22.5-23.5)		(6.0-6.9)		(7.8-8.2)		(355-393)		(94-107)		(120.0-134.0)		(0.041-0.090)			
Station 3	22.7	-	6.0	-	7.5	-	319	-	70	-	109.3	-	0.083	0.083	-	-
<i>C. tentans</i>	(22.4-23.5)		(5.4-6.8)		(7.3-7.6)		(311-325)		(64-75)		(102.0-120.0)		(0.003-0.230)			
Station 4	22.9	-	6.0	-	7.6	-	336	-	80	-	118.0	-	0.037	0.037	-	-
<i>C. tentans</i>	(22.5-23.6)		(5.7-6.7)		(7.5-7.8)		(326-359)		(79-82)		(114.0-124.0)		(0.001-0.076)			
Station 5	23.2	-	6.1	-	7.7	-	344	-	85	-	125.3	-	<0.022	<0.022	-	-
<i>C. tentans</i>	(23.0-23.7)		(5.6-6.8)		(7.6-7.9)		(331-372)		(83-87)		(120.0-134.0)		(<0.001-0.051)			

\* mg/L as CaCO<sub>3</sub>



Appendix B  
Toxicity Split-Sample Sediment Characterization: Metals, Pesticides and Solids,  
Jacob's Creek Sediment Test,  
August 4-14, 1995

Parameter	units	Station 2	Station 3	Station 4	Station 5
Aluminum	mg/kg	12000.0	10000.0	12000.0	15000.0
Arsenic	mg/kg	5.7	5.3	6.1	19.0
Cadmium	mg/kg	1.7	3.0	2.0	11.0
Calcium	mg/kg	1800.0	880.0	1400.0	2100.0
Chromium	mg/kg	13.0	16.0	18.0	32.0
Copper	mg/kg	9.8	5.2	12.0	31.0
Iron	mg/kg	17000.0	13000.0	17000.0	25000.0
Lead	mg/kg	8.9	10.0	13.0	21.0
Magnesium	mg/kg	2200.0	1200.0	1500.0	1900.0
Manganese	mg/kg	1100.0	270.0	630.0	1700.0
Mercury	mg/kg	<0.1	<0.1	<0.1	<0.1
Nickel	mg/kg	27.0	14.0	29.0	89.0
Zinc	mg/kg	60.0	40.0	87.0	280.0
Percent Moisture	%	27.0	16.4	20.8	23.4
Aldrin	µg/kg	<10.0	<10.0	<10.0	<10.0
α-BHC	µg/kg	<10.0	<10.0	<10.0	<10.0
β-BHC	µg/kg	<10.0	<10.0	<10.0	<10.0
χ-BHC	µg/kg	<10.0	<10.0	<10.0	<10.0
δ-BHC	µg/kg	<10.0	<10.0	<10.0	<10.0
Chlordane	µg/kg	<10.0	<10.0	<10.0	<10.0
P'P'DDT	µg/kg	<10.0	<10.0	<10.0	<10.0
P'P'DDE	µg/kg	<10.0	<10.0	<10.0	<10.0
P'P'DDD	µg/kg	<10.0	<10.0	<10.0	<10.0
Dieldrin	µg/kg	<10.0	<10.0	<10.0	<10.0
α-Endosulfan	µg/kg	<10.0	<10.0	<10.0	<10.0
β-Endosulfan	µg/kg	<10.0	<10.0	<10.0	<10.0
Endrin	µg/kg	<10.0	<10.0	<10.0	<10.0
Endrin aldehyde	µg/kg	<10.0	<10.0	<10.0	<10.0
Heptachlor	µg/kg	<10.0	<10.0	<10.0	<10.0
Heptachlor epoxide	µg/kg	<10.0	<10.0	<10.0	<10.0
PCB-1242	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1254	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1221	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1232	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1248	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1260	µg/kg	<25.0	<25.0	<25.0	<25.0
PCB-1016	µg/kg	<25.0	<25.0	<25.0	<25.0
Toxaphene	µg/kg	<500.0	<500.0	<500.0	<500.0
Porewater Ammonia	µg/L	270.0	31.0	97.0	85.0



Appendix B (Continued)  
Toxicity Split-Sample Sediment Characterization: Metals, Pesticides and Solids,  
Jacob's Creek Sediment Test,  
August 4-14, 1995

	Particle Size (%)			
	<2.0 mm	<0.5 mm	<0.125 mm	<0.063 mm
Station 2	99.60	98.49	91.84	88.99
Station 3	100.00	98.41	96.46	95.31
Station 4	99.18	97.40	93.77	92.73
Station 5	97.34	92.54	85.46	82.88



Appendix C  
Sediment Characterization, Jacob's Creek Sediment Test, August 1995

Parameter	Station					Station				
	1	2	3	4	5	1	2	3	4	5
<b>Physical</b>										
Moisture, %	59.88	42.72	26.95	33.23	36.84					
Silt, %	62.00	89.30	93.40	95.80	68.80					
Sand, %	38.00	10.70	6.60	4.20	31.20					

**AVS & SEM (wet wt.)**

AVS, umol/g	35.40	28.00	1.65	14.80	3.49
Cd, mg/kg	1.87	1.62	2.71	1.74	10.10
Cu, mg/kg	1.57	3.64	4.77	4.07	19.40
Pb, mg/kg	4.16	7.39	8.84	11.80	16.20
Ni, mg/kg	17.30	10.20	8.72	5.61	50.90
Zn, mg/kg	63.30	35.40	31.10	30.00	192.00
Total	88.20	58.25	56.14	53.22	288.60

**AVS & SEM Dry wt. Conversion:**

AVS, umol/g	88.24	48.88	2.26	22.17	5.53
Cd, mg/kg	4.66	2.83	3.71	2.61	15.99
Cu, mg/kg	3.91	6.35	6.53	6.10	30.72
Pb, mg/kg	10.37	12.90	12.10	17.67	25.65
Ni, mg/kg	43.12	17.81	11.94	8.40	80.59
Zn, mg/kg	157.78	61.80	42.57	44.93	303.99
Total	219.84	101.69	76.85	79.71	456.93

**Whole Sed., umol/g (dry wt.)**

	0.03	0.02	0.03	0.00	0.12
	0.22	0.25	0.21	0.30	0.73
	0.13	0.16	0.12	0.10	0.24
	1.27	0.65	0.42	0.30	2.06
	2.76	1.48	1.15	1.20	5.86
	4.40	2.56	1.93	2.18	9.02

**umol/g Conversion:**

Cd, umol/g (dry wt.)	0.04	0.03	0.03	0.02	0.14
Cu, umol/g (dry wt.)	0.06	0.10	0.10	0.10	0.48
Pb, umol/g (dry wt.)	0.05	0.06	0.06	0.09	0.12
Ni, umol/g (dry wt.)	0.73	0.30	0.20	0.14	1.37
Zn, umol/g (dry wt.)	2.41	0.95	0.65	0.69	4.65

**Partitioning Results ("No Effect" Predictive Criterion)**

TOT SEM umol/g (dry wt.)	3.30	1.44	1.05	1.03	6.77
AVS, umol/g (dry wt.)	88.24	48.88	2.26	22.17	5.53
<b>SEM/AVS</b>	<b>0.04</b>	<b>0.03</b>	<b>0.46</b>	<b>0.05</b>	<b>1.23</b>

**Toxicity Test Results**

(proportional response*)					
Survival					
<i>H. azteca</i> :	1.00	0.96	1.04	1.01	
<i>C. tentans</i> :	1.13	0.97	1.01	0.97	
Growth					
<i>H. azteca</i> :	0.98	0.79	0.87	0.97	
<i>C. tentans</i> :	0.52	0.59	0.56	0.66	

**Whole Sediment**

TOC, %	1.90	1.06	0.53	0.95	1.40
Cd, mg/kg (wet wt.)	1.40	1.60	2.80	2.00	8.40
Cu, mg/kg (wet wt.)	5.50	9.00	9.80	14.90	29.40
Pb, mg/kg (wet wt.)	10.50	19.00	17.80	20.30	31.90
Ni, mg/kg (wet wt.)	29.80	21.70	17.90	15.30	76.40
Zn, mg/kg (wet wt.)	72.40	55.60	55.00	55.40	242.00
Total, mg/kg (wet wt.)	119.60	106.90	103.30	107.90	388.10

**Interstitial Water (mg/L)**

-	-	-	-	-
nd	nd	nd	nd	nd
nd	nd	nd	nd	nd
nd	nd	nd	nd	nd
nd	nd	0.04	nd	0.01
nd	nd	0.03	0.01	nd
0.00	0.00	0.07	0.01	0.01
7.96	7.94	6.99	7.25	7.73

pH

\*Response compared with formulated control sediment




January 3, 1995

Steven H. Coonrod, WET 1A-M  
Frank J. Sikora, CEB 1C-M

ASSESSING SEDIMENT QUALITY IN RELATION TO METALS AND PAH BIOAVAILABILITY  
IN JACOB'S CREEK AT THE PARADISE FOSSIL PLANT - REPORT OF AUGUST 1995  
TOXICITY/METALS PARTITIONING RESULTS

Page one of the subject report sent to you on December 21, 1995,  
contained a grammatical error. Please replace page one of that report  
with the attached corrected page.



Donald C. Wade  
Senior Toxicologist  
Water Management  
OSA 1B-1

DCW

cc (Attachment):

A. R. Lewis, LP 5D-C  
G. Quintero, CC 1A-C  
R. J. Pryor, WT 10D-K  
D. W. Robinson, LP 5H-C  
D. J. Simbeck, TTL 1A-BFN  
R. K. Tapp, PAF 1A-DRK  
Files, WM, CST 16D-C



## STANDARD REPORT FORM

### TOXICITY BIOMONITORING *HYALELLA AZTECA* (AMPHIPOD), AND *CHIRONOMUS TENTANS* (MIDGE)

Test Title: Jacob's Creek Sediment Toxicity Evaluation of EPA's Metals Partitioning AVS/SEM  
"No Effects" Criteria, Paradise Fossil Plant

Principal Investigator: Damien J. Simbeck

Report Prepared: Donald C. Wade and Damien J. Simbeck

Starting Date: August 4, 1995

Ending Date: August 14, 1995

#### 1.0 EXECUTIVE SUMMARY

Current Environmental Protection Agency (EPA) studies are pursuing the development and use of sediment quality criteria under authority of the Clean Water Act to protect the chemical, physical, and biological integrity of the country's water resources. These criteria are intended to be used to prevent clean sediments from becoming contaminated and to assist in making regulatory and remediation decisions on sediments that are already contaminated. Sediment quality criteria are the EPA's best recommendation of the concentrations of substances in sediment that will not unacceptably affect benthic organisms. [1]

This study was conducted to evaluate applicability of EPA's "no effects" sediment quality criteria for metals at the Tennessee Valley Authority's (TVA) Paradise Fossil Plant (PAF), Drakesboro, Kentucky. The study was designed to determine amounts of toxic metals present in sediments of Jacob's Creek, a tributary stream to the Green River that receives waste water discharges from the plant's ash pond, and determine if those metals were bioavailable. [2] Bioavailability was determined through chemical analyses, based on partitioning of toxic metals (Ni, Zn, Pb, Cu, and Cd) with acid volatile sulfides (AVS), measurements of toxic metal concentrations in sediment interstitial water, and also biologically, based on acute whole-sediment toxicity tests.

This report concludes the biological portion of the study. Two EPA sediment test species, an amphipod, *Hyaella azteca* and a midge, *Chironomus tentans* were exposed to sediments collected from Jacob's Creek at one station upstream from the PAF ashpond discharge and three stations downstream from the ash pond's discharge. Sediment toxicity was evaluated based on lethal and sub-lethal (growth) responses of these organisms.

Ten day exposure to Jacob's Creek sediments resulted in no survival effects to either test organism when compared to sediment at the upstream station or a control sediment formulated as prescribed in EPA sediment test methods. [3] Amphipod growth (dry weight) was statistically reduced in sediments from Stations 3 and 4 downstream from the ashpond discharge compared to both the formulated control sediment (MSD=12.3%) and sediment collected at Station 2, upstream from the ashpond discharge (MSD=11.3%). However, amphipod growth reductions were, in all cases, less than 25 percent and the reduction at station 4 compared with the upstream station was slightly less than the MSD, though

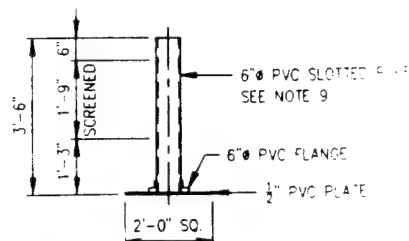
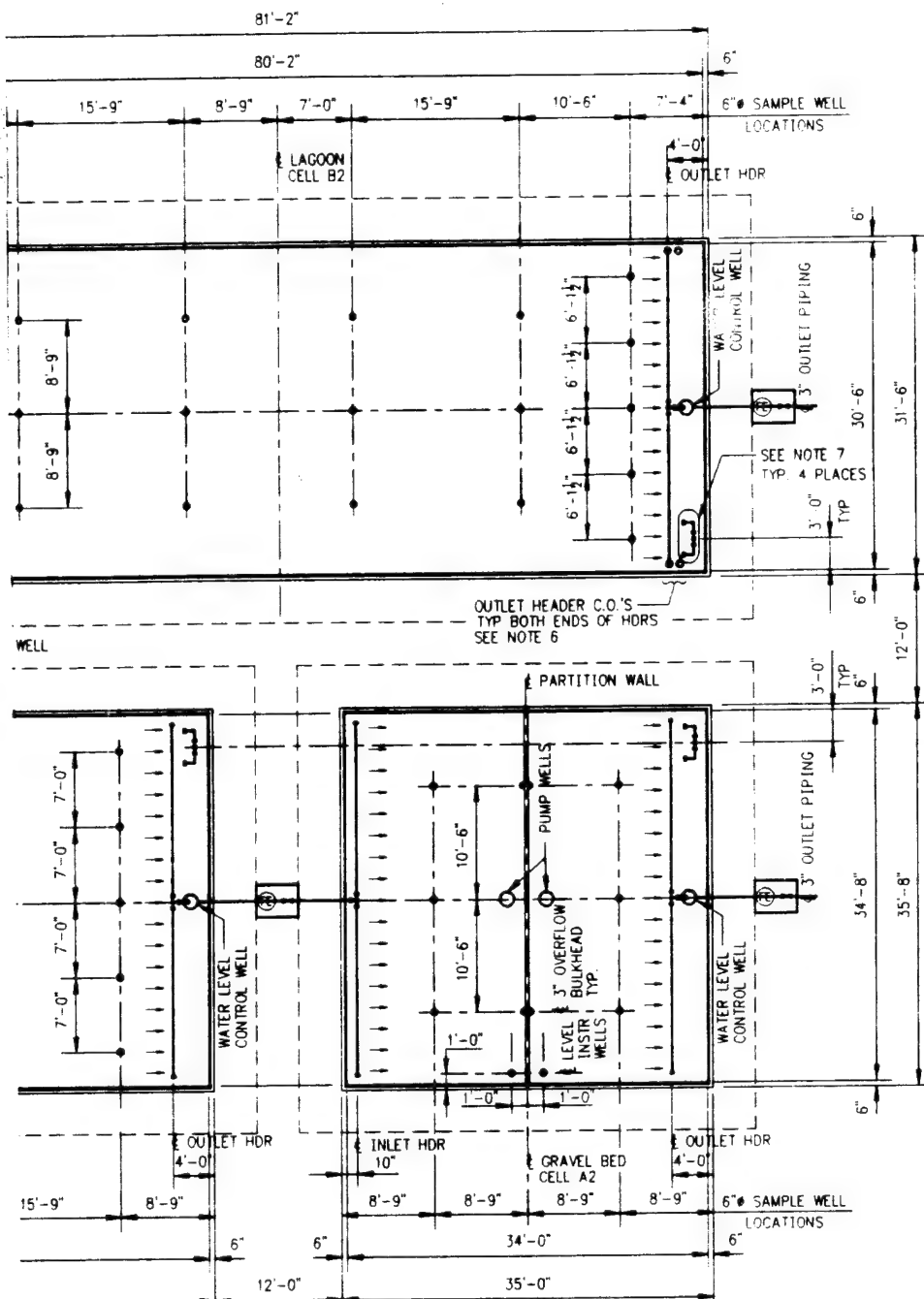


## **Appendix D – FIELD DEMONSTRATION CONSTRUCTION DRAWINGS**



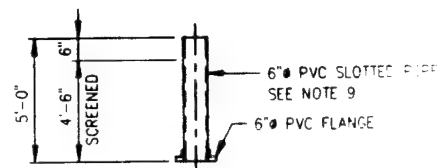






**WELL DETAIL "B"**

34 REQUIRED FOR "B" CELLS SAMPLE WELLS  
2 REQD FOR "B" CELLS LEVEL INSTR WELLS  
 $\frac{1}{2}" = 1'-0"$



**WELL DETAIL "A"**

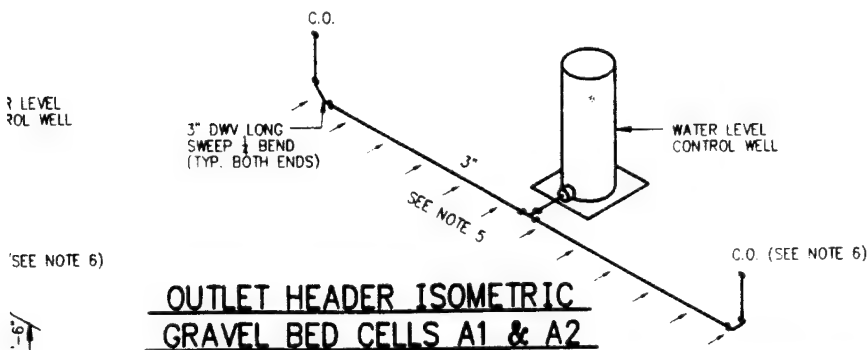
23 REQUIRED FOR "A" CELLS SAMPLE WELLS  
2 REQD FOR "A" CELLS LEVEL INSTR WELLS  
N.T.S.

**NOTES:**

- 1) EXCAVATED SOIL TO BE STOCKPILED FOR FINAL BACKFILL BETWEEN A AND B CELLS.
- 2) SEE DWG D23171 FOR CELL CONSTRUCTION DETAILS
- 3) ALL PIPING SHALL BE SCH 40 PVC UNLESS NOTED.
- 4) ALL PVC PIPE CONNECTIONS TO BE SOLVENT WELDED FOLLOWING MANUFACTURERS INSTALLATION INSTRUCTIONS FOR DEBURRING, CLEANING, PRIMER AND CEMENT
- 5) 3" PVC INLET AND 3" PVC OUTLET HEADERS TO HAVE  $\frac{1}{2}"$  HOLES DRILLED ON 2'-0" CENTERS, ALONG HORIZONTAL CENTERLINE OF ENTIRE HEADER LENGTH
- 6) INLET AND OUTLET HEADER CLEANOUTS TO BE LOCATED FLUSH WITH TOP OF POLY WALL.
- 7) LEAK DETECTION COLUMN LOCATED AS SHOWN SEE FAB AND INSTALLATION DETAILS ON DWG. D23171
- 8) LAGOON OUTLET HEADER SUPPORTS TO BE 3" PVC PIPE, COPEL BOTH ENDS TO FIT SNUGLY BETWEEN THE HORIZONTAL HEADERS. SOLVENT WELD SUPPORTS INTO PLACE.
- 9) 6" PVC SLOTTED "WELL SCREEN" PIPE, TO HAVE  $\frac{1}{8}"$  WIDTH SLOTS ON  $\frac{1}{2}"$  CENTERS.

**REFERENCE DRAWINGS:**

D23169 - SITE LOCATION PLAN  
D23171 - CELL CONSTRUCTION DETAILS  
D23172 - G.A.C. PIT DETAILS  
D23173 - SOIL SAMPLE LOCATION PLAN  
D23175 - BOAT DECKS AND OVERFLOW PIPING



**OUTLET HEADER ISOMETRIC  
GRAVEL BED CELLS A1 & A2**



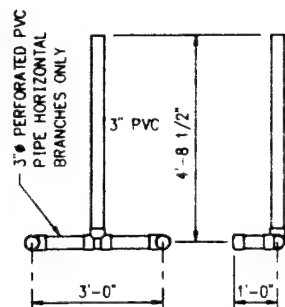
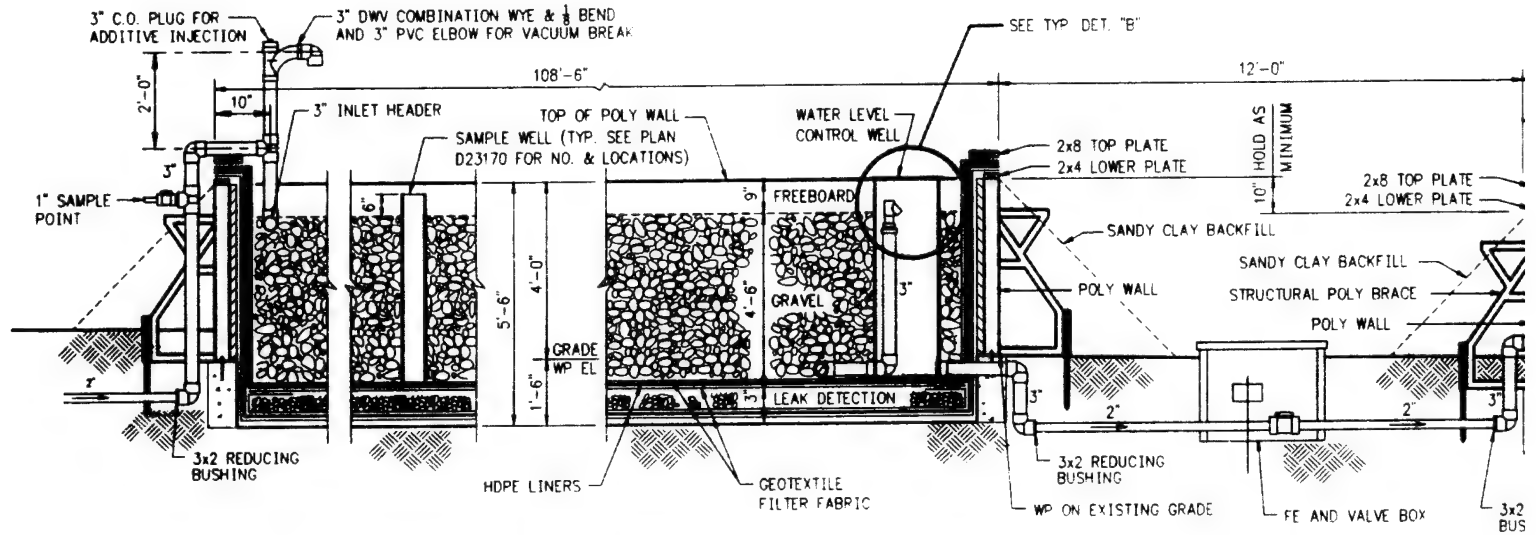
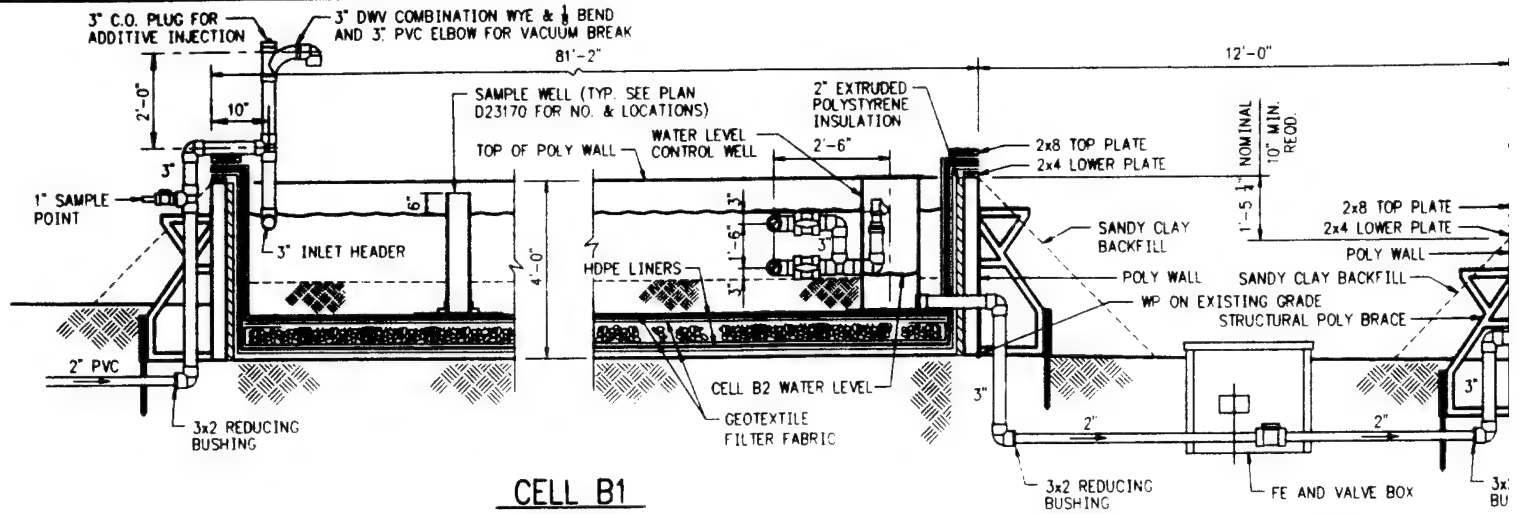
**WESTERN ENGINEERING  
DESIGN**  
MUSCLE SHOALS, ALABAMA

**MILAN ARMY AMMUNITION PLANT  
CONSTRUCTED WETLAND PROJECT  
CELL PIPING PLAN AND DETAILS**

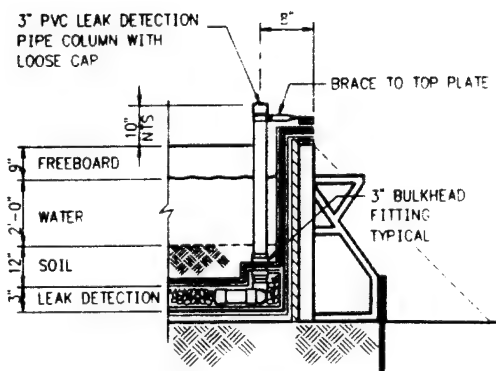
L. E. HALE	1/25/98	R. S. JAMES	1/25/98	SCALE	1/8"=1'-0"
DRAWN	DATE	APPROVED	DATE	WORK	WETLANDS
CHECKED	DATE	APPROVED	DATE	CADD FILE NAME	- DRAWING NO
BILL OF MATERIALS	SHEETS	APPROVED	DATE	<b>D23170 R1</b>	

R1	AS CONSTRUCTED	WETLANDS	LEH	R10	04	03	03
DATE	DESCRIPTION	WORK ORDER	MADE	CHECKED	APPROVED	APPROVED	DATE





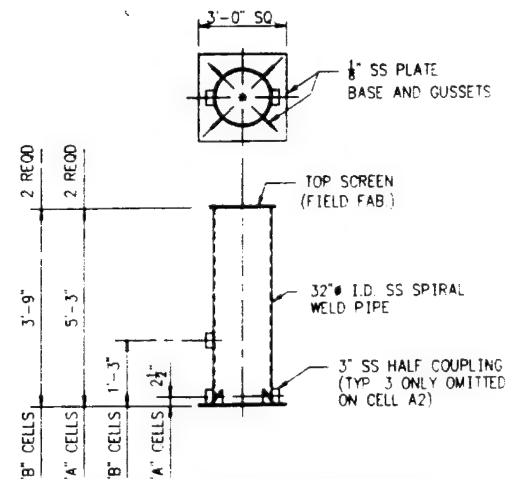
**LEAK DETECTION COLUMN**



**INSTALLATION DETAIL**

**LEAK DETECTION SYSTEM**

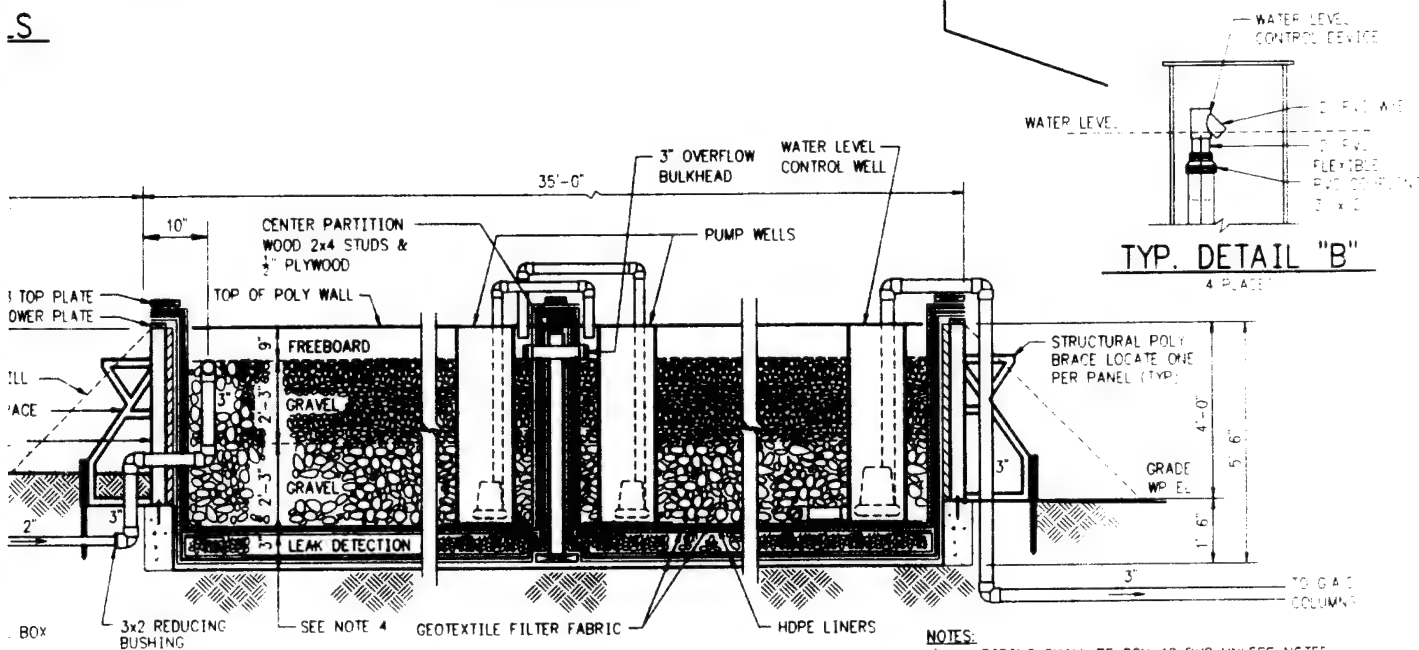
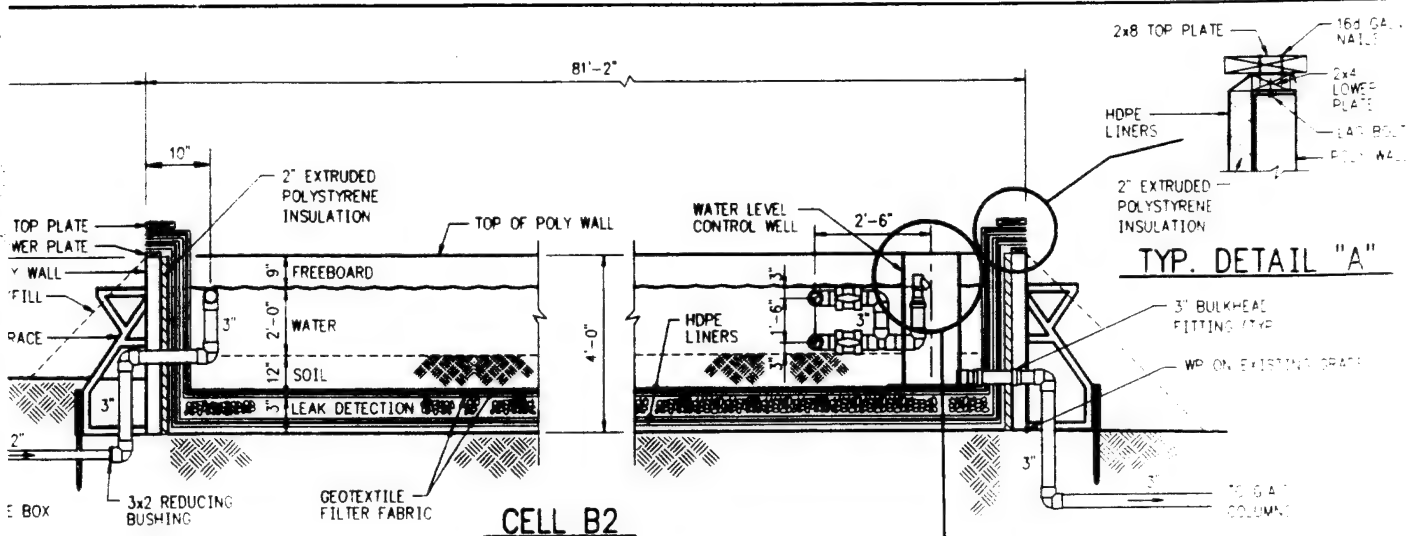
4 REQUIRED



**WATER LEVEL CONTROL**

4 UNITS RECD (AS NOTED)





#### NOTES:

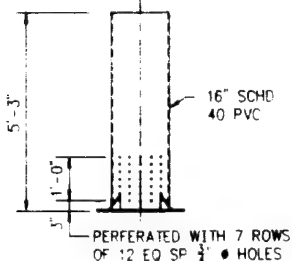
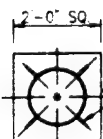
- 1) ALL PIPING SHALL BE SCH 40 PVC UNLESS NOTED
- 2) ALL PVC CONNECTIONS TO BE SOLVENT WELDED ACCORDING TO MANUFACTURERS INSTALLATION INSTRUCTIONS
- 3) ALL HDPE LINER MATERIAL TO BE A MINIMUM 20 MIL THICK LINERS TO BE SEAMLESS, WITH ALL PENETRATIONS SEALED ACCORDING TO MANUFACTURERS INSTALLATION INSTRUCTIONS
- 4) GRAVEL FOR CELL A2 SHALL BE AS FOLLOWS  
LEAK DETECTION: 1/2" - 1" WASHED RIVER GRAVEL  
LOWER FILL: 2" - 3" WASHED LESTONE  
UPPER FILL: 1/2" - 1" WASHED RIVER GRAVEL
- 5) GRAVEL FOR CELL A1 TO BE 1/2" - 1" WASHED RIVER GRAVEL EXCEPT FOR AREAS AROUND INLET AND OUTLET HEADERS THERE THE FILL MUST BE 2" - 3" WASHED LESTONE
- 6) GRAVEL FOR ALL LEAK DETECTION AREAS TO BE 1/2" - 1" WASHED RIVER GRAVEL

#### NOTES: (CONTINUED)

- 7) SECURE PANEL AT BOTTOM TO CONCRETE FOOTER WITH 3/8" EXPANSION ANCHORS IN PREDRILLED HOLES
- 8) INSTALL STRUCTURAL POLY BRACES ONE PER PANEL AND FASTEN PANELS WITH POLY BOLTS PER MANUFACTURERS INSTRUCTIONS

#### REFERENCE DRAWINGS:

- D23169 - SITE LOCATION PLAN  
D23170 - CELL PIPING PLAN AND DETAILS  
D23172 - G.A.C. PIT DETAILS  
D23173 - SOIL SAMPLE LOCATION PLAN  
D23175 - BOAT DECKS AND OVERFLOW PIPING



#### PUMP WELLS

2 UNITS REQD



WESTERN ENGINEERING  
DESIGN  
MUSCLE SHOALS, ALABAMA

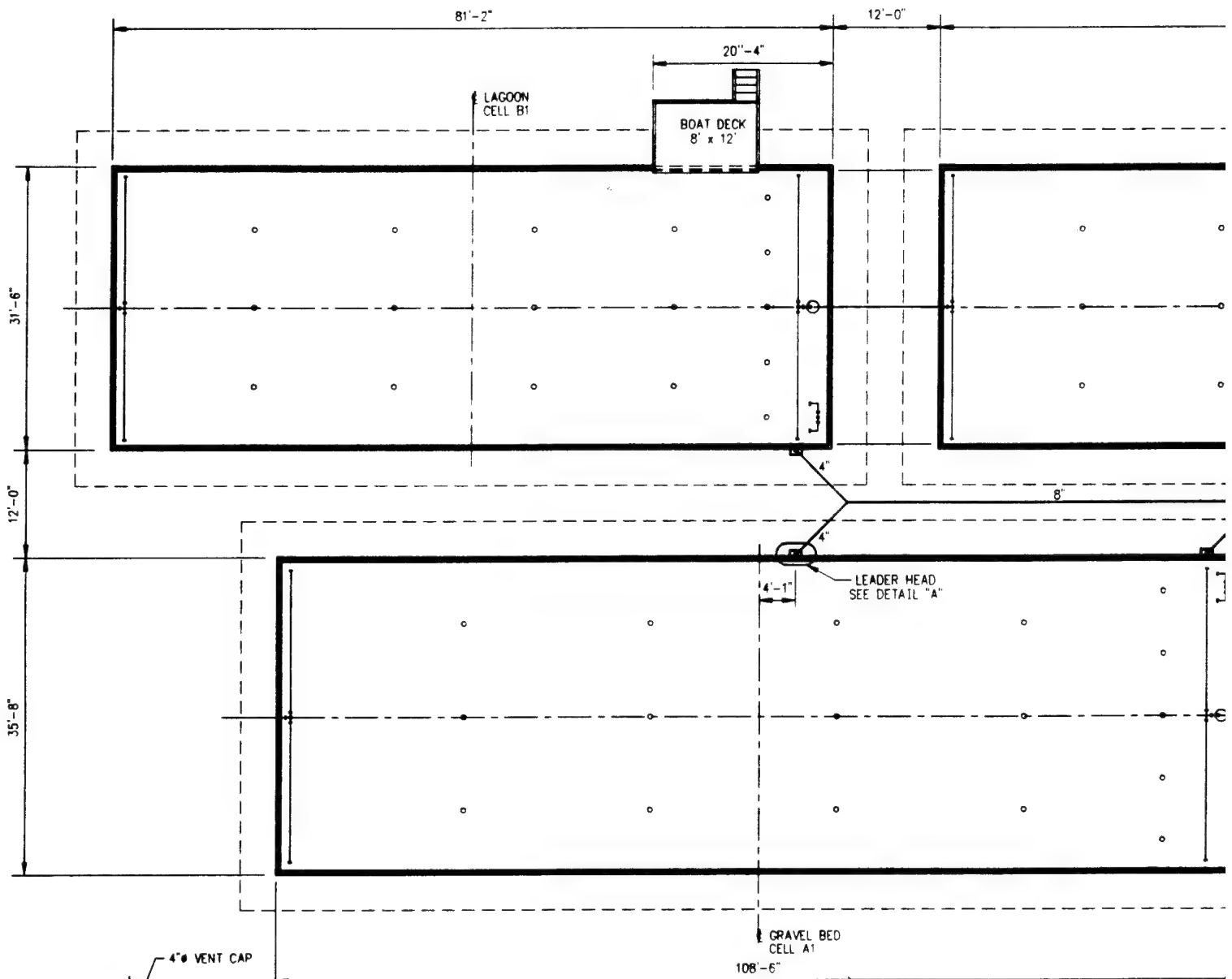
#### MILAN ARMY AMMUNITION PLANT CONSTRUCTED WETLAND PROJECT CELL CONSTRUCTION DETAILS

L. E. MALE	1/22/96	R. S. Summers	25/96	SCALE: 1" = 2'-0"
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CHECKED	DATE	DATE	DATE	DATE
BL. OF MATERIALS	SHEETS	DATE	DATE	DATE

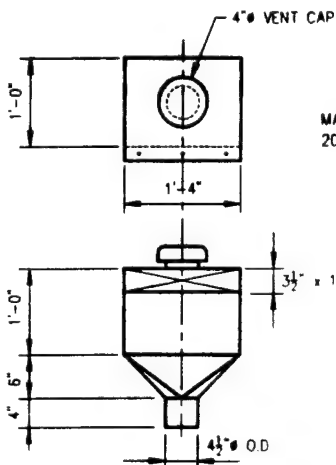
R1	AS CONSTRUCTED	WETLANDS	LEH	ZKS	DA	SH	PA
ISSUE	DESCRIPTION	WORK ORDER	DATE	CHECKED	APPROVED	APPROVED	DATE

D23171 R1

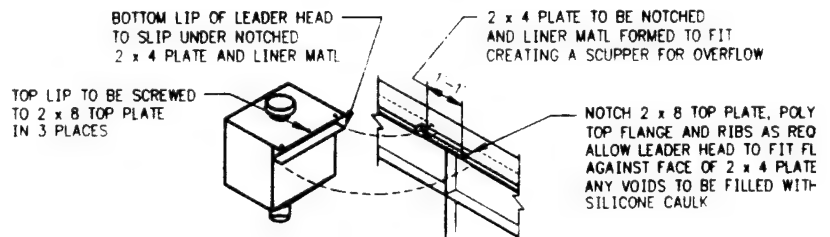
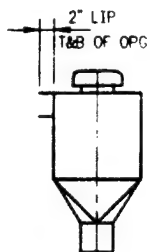




PLAN



MATERIAL OF CONSTRUCTION:  
20GA. GALV. SHEET METAL



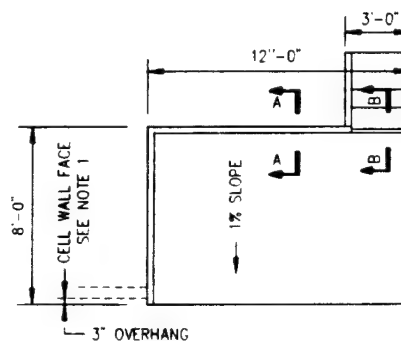
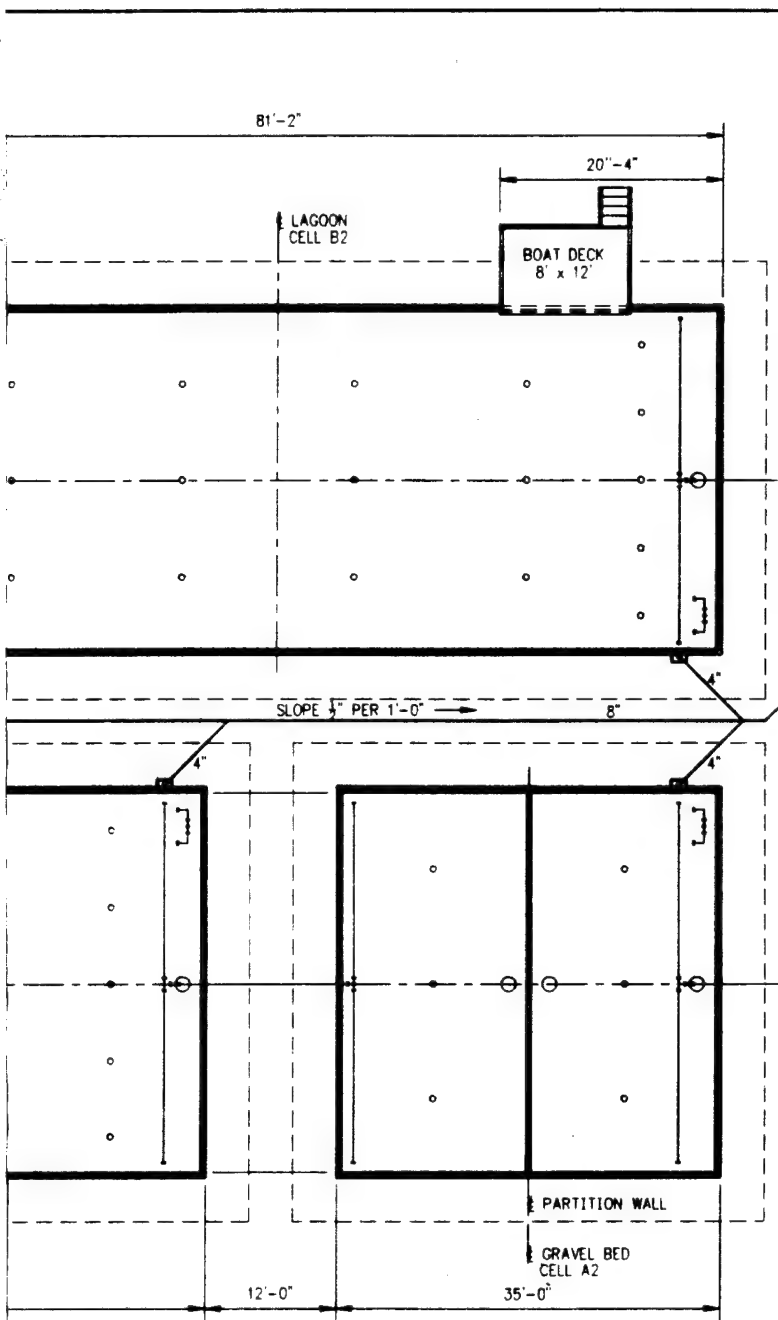
LEADER HEAD - DETAIL "A"

TYPICAL - 5 REQUIRED  
1" = 1'-0"

LEADER HEAD - ISOMETRIC

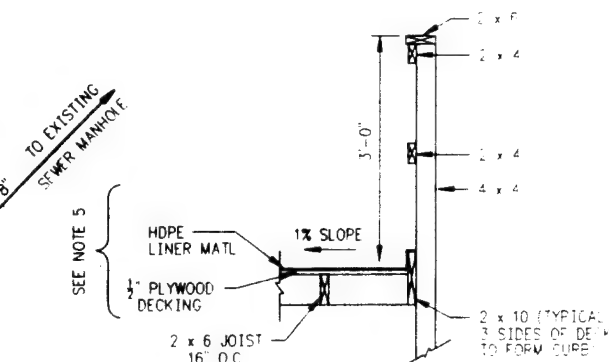
NTS





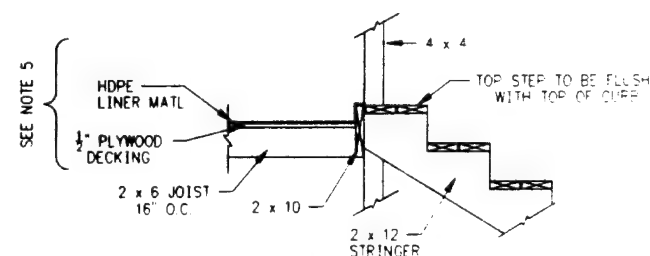
### BOAT DECK - PLAN

TYPICAL - TWO REQUIRED  
 $\frac{1}{4}" = 1'-0"$



### SECTION "A-A"

$\frac{1}{4}" = 1'-0"$



### SECTION "B-B"

$\frac{1}{4}" = 1'-0"$

#### NOTES:

- 1) DECK STRUCTURES MUST NOT BEAR ON OR BE BRACED FROM CELL WALLS.
- 2) DECK WOOD SHALL BE WOLMANIZED PRESSURE TREATED No. 2 SOUTHERN PINE, EXCEPT FOR PLYWOOD DECKING, WHICH SHALL BE EXTERIOR GRADE.
- 3) WOOD DECK STRUCTURES TO BE LEFT UNPAINTED AND ALLOWED TO WEATHER NATURALLY.
- 4) WOOD DECK CONSTRUCTION SHALL MEET ALL LOCAL BUILDING AND SAFETY CODES AND REQUIREMENTS FOR EXTERIOR DECKS, STAIRS AND RAILINGS.
- 5) ALL DECK FRAMING FASTENERS TO BE HOT DIPPED GALVANIZED. HDPE LINER TO BE FASTENED WITH EXTERIOR GRADE "LIQUID NAILS" ALL-PURPOSE ADHESIVE OR EQUAL THIS TO INSURE LINER INTEGRITY.
- 6) ALL PIPING SHALL BE SCH 40 PVC UNLESS NOTED.
- 7) LEADER HEADS TO BE CONNECTED TO DRAIN PIPING WITH A 4" x 4" FLEXIBLE PVC COUPLING.

#### REFERENCE DRAWINGS:

- D23169 - SITE LOCATION PLAN
- D23170 - CELL PIPING PLAN AND DETAILS
- D23171 - CELL CONSTRUCTION DETAILS
- D23172 - G.A.C. PIT DETAILS
- D23173 - SOIL SAMPLE LOCATION PLAN



**WESTERN ENGINEERING  
 DESIGN**  
 MUSCLE SHOALS, ALABAMA

## MILAN ARMY AMMUNITION PLANT CONSTRUCTED WETLAND PROJECT BOAT DECKS & OVERFLOW PIPING

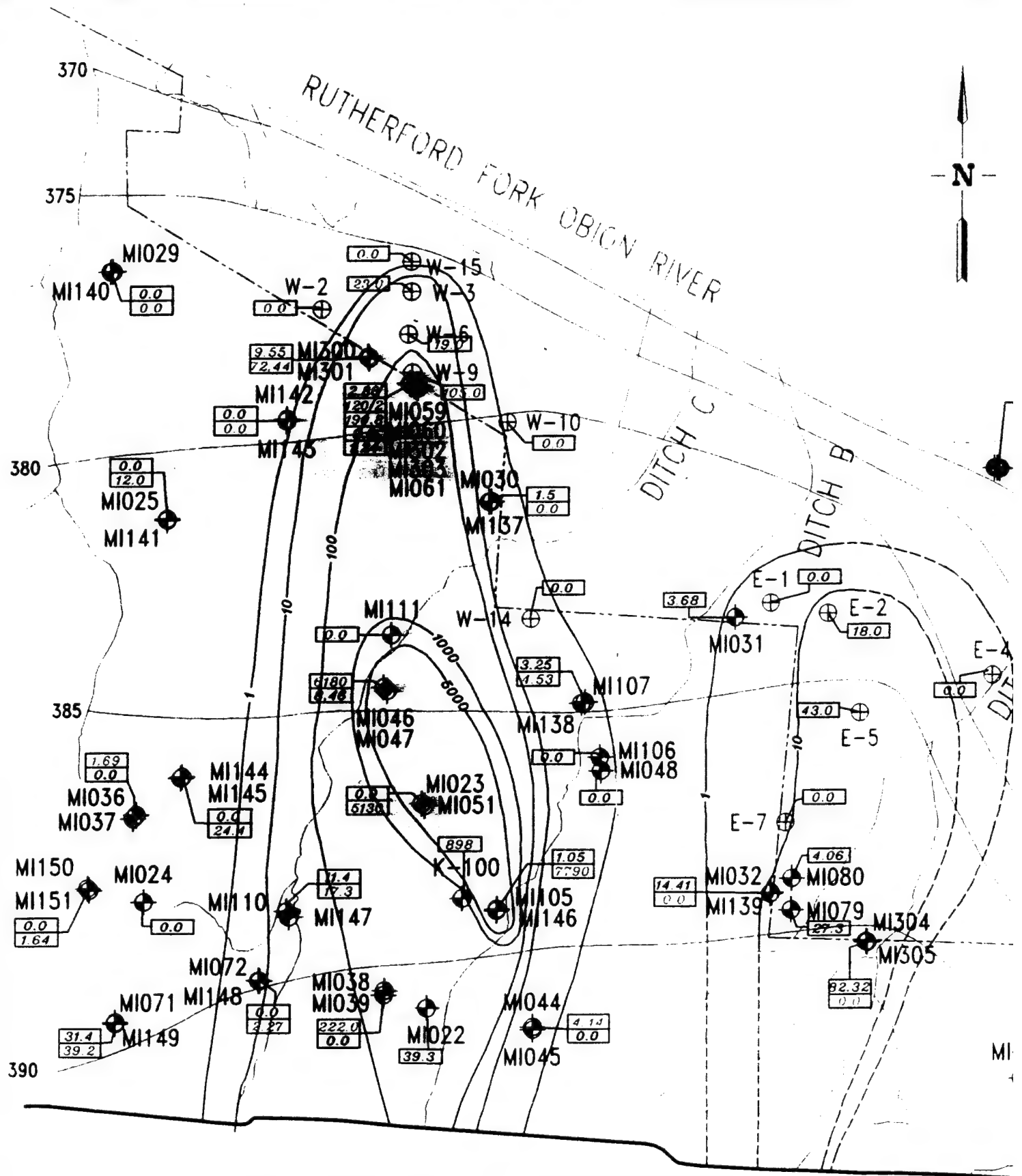
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CHECKED	DATE	APPROVED	DATE	CADD FILE NAME	DRAWING NO.
					D23175 R1

R1	AS CONSTRUCTED	WETLANDS	LEH	res	RL	MS	
ISSUE	DESCRIPTION	WORK ORDER	MADE	CHECKED	APPROVED	APPROVED	DATE

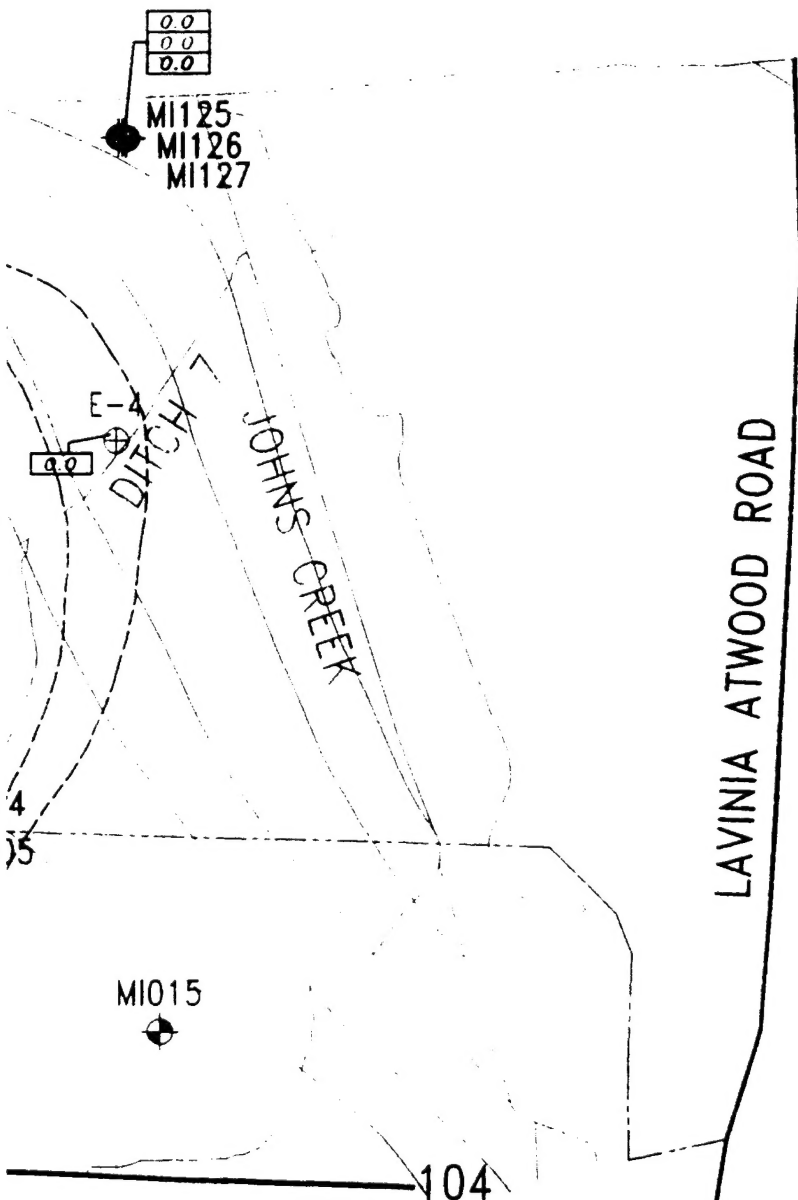
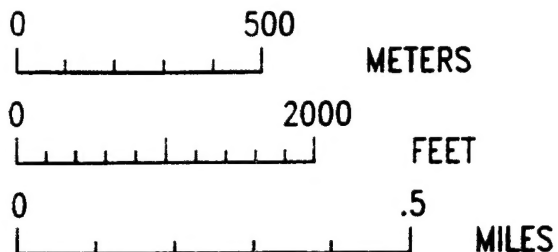
NOTCHED  
 FORMED TO FIT  
 FOR OVERFLOW

2 x 8 TOP PLATE, POLY WALL  
 ANGLE AND RIBS AS REQUIRED TO  
 LEADER HEAD TO FIT FLUSH  
 ST FACE OF 2 x 4 PLATE  
 JOISTS TO BE FILLED WITH  
 CAULK









# LEGEND

- ROAD
- - - - - PROPERTY LINE
- - - - - STREAM/DRAINAGE
- 380 - GROUNDWATER ELEVATION CONTOUR
- 100 - TOTAL EXPLOSIVES ISOPLETH ( $\mu\text{g/L}$ )
- - 10 - ESTIMATED TOTAL EXPLOSIVES ISOPLETH ( $\mu\text{g/L}$ )
- MI300 PERMANENT WELL LOCATION
- ⊕ TEMPORARY WELL LOCATION
- E-5

CONCENTRATION OF TOTAL EXPLOSIVES ( $\mu\text{g/L}$ )

- 100 } SHALLOW WELL
- 100 } INTERMEDIATE WELL
- 100 } DEEP WELL

**FIGURE 2B**  
**TOTAL EXPLOSIVES CONCENTRATION**  
**IN GROUND WATER**  
**AT MAAP NORTHERN BOUNDARY**

CONTRACT NO. DAAA15-81-D-0014		FIGURE 2-1	
<b>KCP KAMMER</b> 1300 Continental Drive Suite 101 Arlington, Maryland 21009 (410) 672-0300		MAP 005 (RPS 02) <b>TOATAL EXPLOSIVES</b> <b>CONCENTRATION IN</b> <b>GROUNDWATER AT</b> <b>NORTHERN</b> <b>BOUNDARY</b>	
PREPARED KDM	DATE 11-18-84	DATE 04-04-85	NORTHBNB
CHECKED JC			



## NOTICE

The construction drawings were being developed at the time this document was being written, but, had not been completed. These drawing will be inserted as they become available.



## Appendix E – REFERENCES



## REFERENCES

1. EPA Annual report, 1994.
2. "Methods for Chemical Analysis of Water and Wastes - Revised March 1983"  
PB84-128677, (U.S.) Environmental Protection Agency, Cincinnati, OH
3. "Preparation Aids for the Development of Category III Quality Assurance Project  
Plans", EPA/600/8-91/005, February 1991, Guy F. Simes, Risk Reduction  
Engineering Laboratory, Office of Research and Development,  
U.S. Environmental Protection Agency, Cincinnati, OH 45268.
4. Title 40, Code of Federal Regulations, Part 136, Appendix A, "Definition and  
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